U.S. PATENT APPLICATION

for

PDE5A CRYSTAL STRUCTURE AND USES

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PDE5A CRYSTAL STRUCTURE AND USES

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims the benefit of Milburn, U.S. Provisional Application 60/444,734, filed February 3, 2003 and Artis et al., U.S. Provisional Application 60/485,627, filed July 7, 2003, all of which are incorporated herein by reference in their entireties, including drawings.

BACKGROUND OF THE INVENTION

[0002] This invention relates to the field of development of ligands for phosphodiesterase 5A (PDE5A) and to the use of crystal structures of PDE5A. The information provided is intended solely to assist the understanding of the reader. None of the information provided nor references cited is admitted to be prior art to the present invention.

[0003] PDEs were first detected by Sutherland and co-workers (Rall, et al., *J. Biol. Chem.*, 232:1065-1076 (1958), Butcher, et al., *J. Biol. Chem.*, 237:1244-1250 (1962)). The superfamily of PDEs is subdivided into two major classes, class I and class II (Charbonneau, H., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, Beavo, J., and Houslay, M.D., eds) 267-296 John Wiley & Sons, Inc., New York (1990)), which have no recognizable sequence similarity. Class I includes all known mammalian PDEs and is comprised of 11 identified families that are products of separate genes (Beavo, et al., *Mol. Pharmacol.*, 46:399-405 (1994); Conti, et al., *Endocr. Rev.*, 16:370-389 (1995); Degerman, et al., *J. Biol. Chem.*, 272:6823-6826 (1997); Houslay, M.D., *Adv. Enzyme Regul.*, 35:303-338 (1995); Bolger, G.B., *Cell Signal*, 6:851-859 (1994); Thompson, et al, *Adv. Second Messenger Phosphoprotein Res.*, 25:165-184 (1992); Underwood, et al., *J. Pharmacol. Exp. Ther.*, 270:250-259 (1994); Michaeli, et al., *J. Biol. Chem.*, 268:12925-12932 (1993); Soderling, et al., *Proc. Natl. Acad. Sci. U.S.A.*, 95:8991-8996 (1998); Soderling, et al., *J. Biol. Chem.*, 273:15553-15558 (1998); Fisher, et al., *J. Biol. Chem.*, 273:15559-15564 (1998)). Some PDEs are highly specific for

hydrolysis of cAMP (PDE4, PDE7, PDE8), some are highly cGMP-specific (PDE5, PDE6, PDE9), and some have mixed specificity (PDE1, PDE2, PDE3, PDE10).

[0004] All of the characterized mammalian PDEs are dimeric, but the importance of the dimeric structure for function in each of the PDEs is unknown. Each PDE has a conserved catalytic domain of ~270 amino acids with a high degree of conservation (25-30%) of amino acid sequence among PDE families, which is located carboxyl-terminal to its regulatory domain. Activators of certain PDEs appear to relieve the influence of autoinhibitory domains located within the enzyme structures (Sonnenberg, et al., *J. Biol. Chem.*, 270:30989-31000 (1995); Jin, et al., *J. Biol. Chem.*, 267:18929-18939 (1992)).

[0005] PDEs cleave the cyclic nucleotide phosphodiester bond between the phosphorus and oxygen atoms at the 3'-position with inversion of configuration at the phosphorus atom (Goldberg, et al., *J. Biol. Chem.*, 255:10344-10347 (1980); Burgers, et al., *J. Biol. Chem.*, 254:9959-9961 (1979)). This apparently results from an in-line nucleophilic attack by the OH— of ionized H_2O. It has been proposed that metals bound in the conserved metal binding motifs within PDEs facilitate the production of the attacking OH— (Francis, et al., *J. Biol. Chem.*, 269:22477-22480 (1994)). The kinetic properties of catalysis are consistent with a random order mechanism with respect to cyclic nucleotide and the divalent cations(s) that are required for catalysis (Srivastava, et al., *Biochem. J.*, 308:653-658 (1995)). The catalytic domains of all known mammalian PDEs contain two sequences (HX_3 HX_n(E/D)) arranged in tandem, each of which resembles the single Zn^{2+}-binding site of metalloendoproteases such as thermolysin (Francis, et al., *J. Biol. Chem.*, 269:22477-22480 (1994)). PDE5 specifically binds Zn^{2+}, and the catalytic activities of PDE4, PDE5, and PDE6 are supported by submicromolar concentrations of Zn^{2+} (Francis, et al., *J. Biol. Chem.*, 269:22477-22480 (1994); Percival, et al., *Biochem. Biophys. Res. Commun.*, 241:175-180 (1997)). Whether each of the Zn^{2+}-binding motifs binds Zn^{2+} independently or whether the two motifs interact to form a novel Zn^{2+}-binding site is not known. The catalytic mechanism for cleaving phosphodiester bonds of cyclic nucleotides by PDEs may be similar to that of certain proteases for cleaving the amide ester of peptides, but the presence of two Zn^{2+} motifs arranged in tandem in PDEs is unprecedented.

[0006] The group of Sutherland and Rall (Berthet, et al., *J. Biol. Chem.*, 229:351-361 (1957)), in the late 1950s, was the first to realize that at least part of the mechanism(s)

whereby caffeine enhanced the effect of glucagon, a stimulator of adenylyl cyclase, on cAMP accumulation and glycogenolysis in liver involved inhibition of cAMP PDE activity. Since that time chemists have synthesized thousands of PDE inhibitors, including the widely used 3-isobutyl-1-methylxanthine (IBMX). Many of these compounds, as well as caffeine, are non-selective and inhibit many of the PDE families. One important advance in PDE research has been the discovery/design of family-specific inhibitors such as the PDE4 inhibitor, rolipram, and the PDE5 inhibitor, sildenafil.

[0007] Precise modulation of PDE function in cells is critical for maintaining cyclic nucleotide levels within a narrow rate-limiting range of concentrations. Increases in cGMP of 2-4-fold above the basal level will usually produce a maximum physiological response. There are three general schemes by which PDEs are regulated: (*a*) regulation by substrate availability, such as by stimulation of PDE activity by mass action after elevation of cyclic nucleotide levels or by alteration in the rate of hydrolysis of one cyclic nucleotide because of competition by another, which can occur with any of the dual specificity PDEs (*e.g.* PDE1, PDE2, PDE3); (*b*) regulation by extracellular signals that alter intracellular signaling (*e.g.* phosphorylation events, Ca^{2+}, phosphatidic acid, inositol phosphates, protein-protein interactions, etc.) resulting, for example, in stimulation of PDE3 activity by insulin (Degerman, et al., *J. Biol. Chem.*, 272:6823-6826 (1997)), stimulation of PDE6 activity by photons through the transducin system (Yamazaki, et al., *J. Biol. Chem.*, 255:11619-11624 (1980)), which alters PDE6 interaction with this enzyme, or stimulation of PDE1 activity by increased interaction with Ca^{2+}/calmodulin; (*c*) feedback regulation, such as by phosphorylation of PDE1, PDE3, or PDE4 catalyzed by PKA after cAmP elevation (Conti, et al., *Endocr. Rev.*, 16:370-389 (1995); Degerman, et al., *J. Biol. Chem.*, 272:6823-6826 (1997); Gettys, et al., *J. Biol. Chem.* 262:333-339 (1987); Florio, et al, *Biochemistry*, 33:8948-8954 (1994)), by allosteric cGMP binding to PDE2 to promote breakdown of cAMP or cGMP after cGMP elevation, or by modulation of PDE protein levels, such as the desensitization that occurs by increased concentrations of PDE3 or PDE4 following chronic exposure of cells to cAMP-elevating agents (Conti, et al., *Endocr. Rev.*, 16:370-389 (1995), Sheth, et al., *Throm. Haemostasis*, 77:155-162 (1997)) or by developmentally related changes in PDE5 content. Other factors that could influence any of the three schemes outlined above are cellular compartmentalization of PDEs (Houslay, M.D., *Adv. Enzyme Regul.*, 35:303-338 (1995)) effected by covalent

modifications such as prenylation or by specific targeting sequences in the PDE primary structure and perhaps translocation of PDEs between compartments within a cell.

[0008] Within the PDE superfamily, four (PDE2, PDE5, PDE6, and PDE10) of the 10 families contain highly cGMP-specific allosteric (non-catalytic) cGMP-binding sites in addition to a catalytic site of varying substrate specificity. Each of the monomers of these dimeric cGMP-binding PDEs contains two homologous cGMP-binding sites of ~110 amino acids arranged in tandem and located in the amino-terminal portion of the protein (Charbonneau, H., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, Beavo, J., and Houslay, M.D., eds) 267-296 (1990); McAllister-Lucas, et al., *J. Biol. Chem.*, 270:30671-30679 (1995)). In PDE2, binding of the cGMP to these sites stimulates the hydrolysis of cAMP at the catalytic site (Beavo, et al., *Mol. Pharmacol.*, 46:399-405 (1994)). PDE2 hydrolyzed cGMP as well as cAMP, and cGMP hydrolysis is stimulated by cGMP binding at the allosteric sites in accordance with positively cooperative kinetics (Manganiello, et al., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation, and Drug Action*, Beavo, J., and Houslay, M.D., eds, 61-85 John Wiley & Sons, Inc., New York (1990)). This could represent a negative feedback process for regulation of tissue cGMP levels (Manganiello, et al., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation, and Drug Action*, Beavo, J., and Houslay, M.D., eds, 61-85 John Wiley & Sons, Inc., New York (1990)), which occurs in addition to the cross-talk between cyclic nucleotide pathways represented by cGMP stimulation of cAMP breakdown. Binding of cGMP to the allosteric sites of PDE6 has not been shown to affect catalysis, but this binding may modulate the interaction of PDE6 with the regulatory protein, transducin, and the inhibitory γ subunit of PDE6 (Yamazaki, et al., *Adv. Cyclic Nucleotide Protein Phosphorylation Res.*, 16:381-392 (1984)).

[0009] The first recognized cGMP-binding PDE was discovered as a cGMP-binding protein in lung tissue during a search for cyclic nucleotide-binding proteins other than cyclic nucleotide-dependent protein kinases (Lincoln, et al., *Proc. Natl. Acad. Sci U.S.A.*, 73:2559-2563 (1976)). By DEAE-cellulose chromatography, this protein appeared as a "peak 1" cGMP-binding protein that was separated from a "peak 2" cGMP-binding protein, which was shown to be PKG. The peak 1 protein possessed both cGMP-binding as well as a distinct cGMP-specific PDE catalytic activity (Francis, et al., *J. Biol. Chem.*, 255:620-626 (1980)), and it was subsequently named PDE5. Davis and Kuo (Davis, et al.,

J. Biol. Chem., 252:4078-4084 (1977)) also described a cGMP-specific PDE activity in lung tissue, and Hamet and Coquil (Hamet, et al., *J. Cyclic Nucleotide Res.*, 4:281-290 (1978)) characterized a cGMP-binding, cGMP-specific PDE in platelets.

[0010] PDE5 has been purified and cloned (Francis, et al., *J. Biol. Chem.*, 255:620-626 (1980); Francis, et al., *Methods Enzymol.*, 159:722-729 (1988); Thomas, et al., *J. Biol. Chem.*, 265:14964-14970 (1990); McAllister-Lucas, et al., *J. Biol. Chem.*, 268:22863-22873 (1993)). Two alternatively spliced variants of PDE5 have recently been identified (Yanaka, et al., *Eur. J. Biochem.*, 255:391-399 (1998); Loughney, et al., *Gene (Amst.)*., 216:137-147 (1998)). The tissue distribution of PDE5 (subunit M_r ~100,000) commonly coincides with that of PKG. This is probably not fortuitous because both PDE5 and PKG are major intracellular receptors for cGMP, and PKG is an excellent catalyst *in vitro* for phosphorylation of PDE5 (Thomas, et al., *J. Biol. Chem.*, 265:14971-14978 (1990)).

[0011] Evidence regarding the presence of conserved Zn^{2+}-binding motifs (HX_3 HX_n(E/D)) in PDEs and their involvement in catalysis was first demonstrated using PDE5 (Francis, et al., *J. Biol. Chem.*, 269:22477-22480 (1994)). Site-directed mutagenesis confirms the catalytic importance of each residue of these motifs A and B (Turko, et al., *J. Biol. Chem.*, 273:6460-6466 (1998)). Substitution of either of the invariant aspartic acid residues (Asp-714, Asp-754) further downstream in the sequence is also highly deleterious, and each of these residues may participate in the catalytic process perhaps as a catalytic base or as a coordinating ligand for a required metal. The most dramatic increases in K_m for cGMP are caused by site-directed mutagenesis of Tyr-602 and Glu-775. These two residues could form part of the cGMP-binding pocket of the catalytic site of PDE5. Because some mutations affecting k_{cat} and K_m are juxtaposed in the primary sequence, the cGMP-binding pocket and catalytic machinery are likely to involve overlapping subdomains within the catalytic domain of PDE5. All of the components required for catalytic activity of PDE5 are contained within a single monomeric catalytic domain. (Furchgott & Vanhoutte, *FASEB J.* 3:2007-2018 (1997).)

[0012] Occupation of the allosteric cGMP-binding sites of PDE5 is required for specific phosphorylation of Ser-92 by PKG or PKA, and occupation of the binding sites is also associated with an increase in the Stokes radius of the enzyme, implying that a conformational change occurs (Francis, et al., *Methods*, 14:81-92 (1998)). A direct effect

of cGMP binding to the allosteric sites on cGMP breakdown at the catalytic site has not been demonstrated, although the principle of reciprocity (binding of cGMP at the catalytic site stimulates binding at the allosteric sites) dictates that there should be an effect (Weber, G., *Adv. Protein Chem.*, 29:1-83 (1975); Francis, et al., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, Beavo, J., and Houslay, M.D., eds, 117-140, John Wiley & Sons, Inc., New York (1990)). The stimulatory effect of cGMP analogs specific for the catalytic site on cGMP binding to the allosteric site(s) of PDE5 suggests that interaction of cGMP with the catalytic site precedes cGMP binding to the allosteric binding site(s) (Francis, et al., *J. Biol. Chem.*, 255:620-626 (1980); Thomas, et al., *J. Biol. Chem.*, 265:14971-14978 (1990)). This implies that upon cGMP elevation in cells, cGMP breakdown at the catalytic site would increase because of mass action (increased substrate availability). This increased cGMP interaction at the catalytic site would enhance cGMP binding at the allosteric sites, thus increasing phosphorylation of the enzyme to promote further increases in cGMP breakdown. Although experimental results are consistent with such a sequence of events, this pathway has not been proven unequivocally in broken cell systems. However, rapid phosphorylation of PDE5, which is associated with increased PDE activity, occurs in intact tissues in response to stimulation by atrial natriuretic factor and may be caused by PKG action (Wyatt, et al., *Am. J. Physiol.*, 274:H448-H455 (1998)). This process could represent negative-feedback regulation of cGMP levels in cells. PKA can also phosphorylate PDE5 *in vitro*, albeit at about 10% the rate at which PKG catalyzes this reaction; whether or not this occurs *in vivo* is uncertain because the concomitant elevation of cGMP and cAMP would be required to expose Ser-92 and activate PKA, respectively. Burns *et al.* (Burns, et al., *Biochem. J.*, 283:487-491 (1992)) have reported that a partially purified PDE5 from guinea pig lung is activated when phosphorylated by PKA. PDE5 may also be regulated by other low molecular weight factors, and these could alter the effects of phosphorylation (Lochhead, et al., *J. Biol. Chem.*, 272:18397-18403 (1997)). As is the case for PDE4, PDE5 may also be subject to long term regulation through changes in enzyme concentration in some cell types (Sanchez, et al., *Pediatr. Res.*, 43:163-168 (1998); Kotera, et al., *Eur. J. Biochem.*, 249:434-442 (1997); Bakre, et al., *FEBS Lett.*, 408:345-349 (1997)).

[0013] The K_D of PDE5 for binding cGMP in the allosteric sites is ~0.2 μM (Thomas, et al., *J. Biol. Chem.*, 265:14964-14970 (1990)). The presence of two kinetically distinct allosteric cGMP-binding sites in PDE5 was first suggested by the curvilinear pattern of

cGMP dissociation from the enzyme. Studies using site-directed mutagenesis confirm the presence of two sites and indicate that the binding of cGMP to each allosteric site could involve a NK(*X*)$_n$D motif (McAllister-Lucas, et al., *J. Biol. Chem.*, 270:30671-30679 (1995); Turko, et al., *J. Biol. Chem.*, 271:22240-22244 (1996)), which resembles that used by G proteins for binding GTP (Pai, et al, *Nature*, 341:209-214 (1989)). The conserved sequence of the allosteric cyclic nucleotide-binding sites in PDE2, PDE5, PDE6, and PDE10 is evolutionarily distinct from that of the family containing PKG, PKA, and cation channels (McAllister-Lucas, et al., *J. Biol. Chem.*, 268:22863-22873 (1993)), indicating that the allosteric cGMP-binding sites of these PDEs represent a newly recognized class of cyclic nucleotide receptors. Another class may be represented by the catalytic sites of PDEs, the sequences of which contain a binding pocket for cyclic nucleotides in the catalytic domain in order to optimize the catalytic process. In PDE5, classical PDE inhibitors and selected cyclic nucleotide analogs compete with cGMP at the catalytic site but do not interact with the cGMP-binding allosteric sites (Francis, et al., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, Beavo, J., and Houslay, M.D., eds, 117-140, John Wiley & Sons, Inc., New York (1990)). The order of potency of some common PDE inhibitors for PDE5 is sildenafil > zaprinast > dipyridamole > IBMX > cilostamide > theophylline > caffeine > rolipram (Fig. 3) (Thomas, et al., *J. Biol. Chem.*, 265:14964-14970 (1990); Ballard, et al., *J. Urol.*, 159:2164-2171 (1998)). Many cyclic nucleotide analogs are also inhibitors of PDE5 (Francis, et al., *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*, Beavo, J., and Houslay, M.D., eds, 117-140, John Wiley & Sons, Inc., New York (1990)), which is to be expected based on the structural similarity of these compounds with cGMP. Some IBMX analogs modified at the 8-position, such as 8-(2-chlorobenzyl)-IBMX, are more potent inhibitors than are any of the cyclic nucleotide analogs (Sekhar, et al., *Phosphodiesterase Inhibitors*, Schudt, C., Dent, G., and Rabe, K.F., eds, 135-146, Academic Press, New York (1996)). Even though the IBMX analogs are generally better PDE5 inhibitors than are cyclic nucleotide analogs, many of the latter are more potent for relaxing intact vascular smooth muscle.

[0014] Because the PDE inhibitors show competitive kinetics with respect to cGMP in the catalytic site of PDE5, they would be expected to form molecular contacts like those formed by cGMP. However, results of mutagenesis of PDE5 indicate that, although both zaprinast, a potent PDE5 inhibitor, and cGMP appear to make contact with several of the

same amino acids in the catalytic domain, some of the residues that are important for interaction with zaprinast, *e.g.* Asp-754 and Gly-780, are not critical for interaction with cGMP (Turko, et al., *J. Biol. Chem.*, 273:6460-6466 (1998)). As noted above, Asp-754 is crucial for efficient catalysis, which is suggestive that inhibition by zaprinast could be due in part to interference with an important function of Asp-754.

[0015] The PDE5 subfamily has only one member: PDE5A (Corbin and Francis, "Cyclic GMP Phosphodiesterase-5: Target of Sildenafil," *The Journal of Biological Chemistry*, 274(20):13729-13732 (1999)). PDE5 possesses a preference for cGMP over cAMP as a substrate. PDE5 is expressed in smooth muscle tissue (Table 1), importantly in the corpus cavernosum. This enzyme possesses two GAF domains in the N-terminal regulatory region. These GAF domains act in concert to bind cGMP and mediate dimerization and activation PDE activity. A recent crystal structure of the PDE2 GAF domain suggests possible mechanisms by which the GAF domains bind cGMP and mediate dimerization (Martinez et al., *Proc Natl Acad Sci USA* 99:13260-13265 (2002)). PDE5 has attracted considerable attention as a therapeutic target due to the tremendous commercial success of Viagra (Pfizer) (Rotella, 2002, Phosphodiesterase 5 inhibitors: Current status and potential applications, *Nature Reviews* 1:674-682). In addition to Viagra (sildenafil), two other drugs are quite far along in the approval process, namely vardenafil (Bayer) and tadalafil (Lilly/ICOS). One apparent drawback to these compounds is some cross-reactivity with the closely related PDE families PDE6 and PDE11 (Gresser and Gleiter, *Eur J Med Res* 7:435-446 (2002)). The availability of PDE5 structural information may enable the discovery of PDE5 inhibitors with improved selectivity versus PDE6 and PDE11. The crystal structure of PDE5 has not been reported in the literature.

SUMMARY OF THE INVENTION

[0016] The present invention concerns structural information about PDE5A, crystals of PDE5A with and without binding compounds, and the use of the PDE5A crystals and structural information about the PDE5A to develop PDE5A ligands, which can be developed from new chemical classes, or can be developed from previously known PDE5A ligands.

[0017] Thus, in a first aspect, the invention concerns a method for developing ligands binding to a PDE5A, where the method includes identifying as molecular scaffolds one or more compounds that bind to a binding site of PDE5A; determining the orientation of at least one molecular scaffold in co-crystals with PDE5A; identifying chemical structures of one or more of the molecular scaffolds, that, when modified, alter the binding affinity or binding specificity or both between the molecular scaffold and the PDE5A; and synthesizing a ligand in which one or more of the chemical structures of the molecular scaffold is modified to provide a ligand that binds to the PDE5A with altered binding affinity or binding specificity or both.

[0018] The terms "PDE5A phosphodiesterase" and "PDE5A" mean an enzymatically active phosphodiesterase that contains a portion with greater than 90% amino acid sequence identity to amino acid residues 531-875 of native PDE5A as shown in Table 4, for a maximal alignment over an equal length segment; or that contains a portion with greater than 90% amino acid sequence identity to at least 200 contiguous amino acids from amino acid residues 531-875 of native PDE5A or the amino acid sequence provided in Table 2 that retains binding to natural PDE5A ligand cGMP. Preferably the sequence identity is at least 95, 97, 98, 99, or even 100%. Preferably the specified level of sequence identity is over a sequence at least 300 contiguous amino acid residues.

[0019] The term "PDE5A phosphodiesterase domain" refers to a reduced length PDE5A (*i.e.*, shorter than a full-length PDE5A by at least 100 amino acids that includes the phosphodiesterase catalytic region in PDE5A. Highly preferably for use in this invention, the phosphodiesterase domain retains phosphodiesterase activity, preferably at least 50% the level of phosphodiesterase activity as compared to the native PDE5A, more preferably at least 60, 70, 80, 90, or 100% of the native activity.

[0020] As used herein, the terms "ligand" and "modulator" are used equivalently to refer to a compound that modulates the activity of a target biomolecule, *e.g.*, an enzyme such as a kinase or phosphodiesterase. Generally a ligand or modulator will be a small molecule, where "small molecule refers to a compound with a molecular weight of 1500 daltons or less, or preferably 1000 daltons or less, 800 daltons or less, or 600 daltons or less. Thus, an "improved ligand" is one that possesses better pharmacological and/or pharmacokinetic properties than a reference compound, where "better" can be defined by a

person for a particular biological system or therapeutic use. In terms of the development of ligands from scaffolds, a ligand is a derivative of a scaffold.

[0021] In the context of binding compounds, molecular scaffolds, and ligands, the term "derivative" or "derivative compound" refers to a compound having a chemical structure that contains a common core chemical structure as a parent or reference compound, but differs by having at least one structural difference, *e.g.*, by having one or more substituents added and/or removed and/or substituted, and/or by having one or more atoms substituted with different atoms. Unless clearly indicated to the contrary, the term "derivative" does not mean that the derivative is synthesized using the parent compound as a starting material or as an intermediate, although in some cases, the derivative may be synthesized from the parent.

[0022] Thus, the term "parent compound" refers to a reference compound for another compound, having structural features continued in the derivative compound. Often but not always, a parent compound has a simpler chemical structure than the derivative.

[0023] By "chemical structure" or "chemical substructure" is meant any definable atom or group of atoms that constitute a part of a molecule. Normally, chemical substructures of a scaffold or ligand can have a role in binding of the scaffold or ligand to a target molecule, or can influence the three-dimensional shape, electrostatic charge, and/or conformational properties of the scaffold or ligand.

[0024] The term "binds" in connection with the interaction between a target and a potential binding compound indicates that the potential binding compound associates with the target to a statistically significant degree as compared to association with proteins generally (*i.e.*, non-specific binding). Thus, the term "binding compound" refers to a compound that has a statistically significant association with a target molecule. Preferably a binding compound interacts with a specified target with a dissociation constant (k_d) of 1 mM or less. A binding compound can bind with "low affinity", "very low affinity", "extremely low affinity", "moderate affinity", "moderately high affinity", or "high affinity" as described herein.

[0025] In the context of compounds binding to a target, the term "greater affinity" indicates that the compound binds more tightly than a reference compound, or than the

same compound in a reference condition, *i.e.*, with a lower dissociation constant. In particular embodiments, the greater affinity is at least 2, 3, 4, 5, 8, 10, 50, 100, 200, 400, 500, 1000, or 10,000-fold greater affinity.

[0026] Also in the context of compounds binding to a biomolecular target, the term "greater specificity" indicates that a compound binds to a specified target to a greater extent than to another biomolecule or biomolecules that may be present under relevant binding conditions, where binding to such other biomolecules produces a different biological activity than binding to the specified target. Typically, the specificity is with reference to a limited set of other biomolecules, *e.g.*, in the case of PDE5A, other phosphodiesterases (*e.g.*, PDE1, PDE6, and/or PDE11) or even other type of enzymes. In particular embodiments, the greater specificity is at least 2, 3, 4, 5, 8, 10, 50, 100, 200, 400, 500, or 1000-fold greater specificity.

[0027] As used in connection with binding of a compound with PDE5A, the term "interact" indicates that the distance from a bound compound to a particular amino acid residue will be 5.0 angstroms or less. In particular embodiments, the distance from the compound to the particular amino acid residue is 4.5 angstroms or less, 4.0 angstroms or less, or 3.5 angstroms or less. Such distances can be determined, for example, using co-crystallography, or estimated using computer fitting of a compound in a PDE5A active site.

[0028] For reference to particular amino acid residues in PDE5A, polypeptide residue number is defined by the numbering provided in Yanaka et al., 1998, *Eur. J. Biochem.* 255:391-399.

[0029] In a related aspect, the invention provides a method for developing ligands specific for PDE5A, where the method involves determining whether a derivative of a compound that binds to a plurality of phosphodiesterases (e.g., a molecular scaffold) has greater specificity for the PDE5A phosphodiesterase than the parent compound with respect to other phosphodiesterases.

[0030] As used herein in connection with binding compounds or ligands, the term "specific for PDE5A phosphodiesterase", "specific for PDE5A" and terms of like import mean that a particular compound binds to PDE5A to a statistically greater extent than to

other phosphodiesterases that may be present in a particular organism. Also, where biological activity other than binding is indicated, the term "specific for PDE5A" indicates that a particular compound has greater biological activity associated with binding PDE5A than to other phosphodiesterases. Preferably, the specificity is also with respect to other biomolecules (not limited to phosphodiesterases) that may be present from an organism.

[0031] In another aspect, the invention provides a method for obtaining improved ligands binding to PDE5A, where the method involves identifying a compound that binds to PDE5A, determining whether that compound interacts with one or more conserved PDE5A active site residues, and determining whether a derivative of that compound binds to the PDE5A with greater affinity or greater specificity or both than the parent binding compound. Binding with greater affinity or greater specificity or both than the parent compound indicates that the derivative is an improved ligand. This process can also be carried out in successive rounds of selection and derivatization and/or with multiple parent compounds to provide a compound or compounds with improved ligand characteristics. Likewise, the derivative compounds can be tested and selected to give high selectivity for PDE5A, or to give cross-reactivity to a particular set of targets, for example to a subset of phosphodiesterases that includes PDE5A. In particular embodiments, known PDE5A inhibitors can be used, and derivatives with greater affinity and/or greater specificity can be developed, preferably using PDE5A structure information; greater specificity for PDE5A relative to PDE1, PDE6, and/or PDE11 is developed.

[0032] By "molecular scaffold" or "scaffold" is meant a simple target binding molecule to which one or more additional chemical moieties can be covalently attached, modified, or eliminated to form a plurality of molecules with common structural elements. The moieties can include, but are not limited to, a halogen atom, a hydroxyl group, a methyl group, a nitro group, a carboxyl group, or any other type of molecular group including, but not limited to, those recited in this application. Molecular scaffolds bind to at least one target molecule, preferably to a plurality of molecules in a protein family, and the target molecule can preferably be a enzyme, receptor, or other protein. Preferred characteristics of a scaffold can include binding at a target molecule binding site such that one or more substituents on the scaffold are situated in binding pockets in the target molecule binding site; having chemically tractable structures that can be chemically modified, particularly by synthetic reactions, so that a combinatorial library can be easily constructed; having

chemical positions where moieties can be attached that do not interfere with binding of the scaffold to a protein binding site, such that the scaffold or library members can be modified to form ligands, to achieve additional desirable characteristics, *e.g.*, enabling the ligand to be actively transported into cells and/or to specific organs, or enabling the ligand to be attached to a chromatography column for additional analysis. Thus, a molecular scaffold is an identified target binding molecule prior to modification to improve binding affinity and/or specificity, or other pharmacalogic properties.

[0033] The term "scaffold core" refers to the core structure of a molecular scaffold onto which various substituents can be attached. Thus, for a number of scaffold molecules of a particular chemical class, the scaffold core is common to all the scaffold molecules. In many cases, the scaffold core will consist of or include one or more ring structures.

[0034] By "binding site" is meant an area of a target molecule to which a ligand can bind non-covalently. Binding sites embody particular shapes and often contain multiple binding pockets present within the binding site. The particular shapes are often conserved within a class of molecules, such as a molecular family. Binding sites within a class also can contain conserved structures such as, for example, chemical moieties, the presence of a binding pocket, and/or an electrostatic charge at the binding site or some portion of the binding site, all of which can influence the shape of the binding site.

[0035] By "binding pocket" is meant a specific volume within a binding site. A binding pocket can often be a particular shape, indentation, or cavity in the binding site. Binding pockets can contain particular chemical groups or structures that are important in the non-covalent binding of another molecule such as, for example, groups that contribute to ionic, hydrogen bonding, or van der Waals interactions between the molecules.

[0036] By "orientation", in reference to a binding compound bound to a target molecule is meant the spatial relationship of the binding compound (which can be defined by reference to at least some of its consitituent atoms) to the binding pocket and/or atoms of the target molecule at least partially defining the binding pocket.

[0037] In the context of target molecules in this invention, the term "crystal" refers to a regular assemblage of a target molecule of a type suitable for X-ray crystallography. That is, the assemblage produces an X-ray diffraction pattern when illuminated with a beam of

X-rays. Thus, a crystal is distinguished from an aggolmeration or other complex of target molecule that does not give a diffraction pattern.

[0038] By "co-crystal" is meant a complex of the compound, molecular scaffold, or ligand bound non-covalently to the target molecule and present in a crystal form appropriate for analysis by X-ray or protein crystallography. In preferred embodiments the target molecule-ligand complex can be a protein-ligand complex.

[0039] The phrase "alter the binding affinity or binding specificity" refers to changing the binding constant of a first compound for another, or changing the level of binding of a first compound for a second compound as compared to the level of binding of the first compound for third compounds, respectively. For example, the binding specificity of a compound for a particular protein is increased if the relative level of binding to that particular protein is increased as compared to binding of the compound to unrelated proteins.

[0040] As used herein in connection with test compounds, binding compounds, and modulators (ligands), the term "synthesizing" and like terms means chemical synthesis from one or more precursor materials.

[0041] The phrase "chemical structure of the molecular scaffold is modified" means that a derivative molecule has a chemical structure that differs from that of the molecular scaffold but still contains common core chemical structural features. The phrase does not necessarily mean that the molecular scaffold is used as a precursor in the synthesis of the derivative.

[0042] By "assaying" is meant the creation of experimental conditions and the gathering of data regarding a particular result of the experimental conditions. For example, enzymes can be assayed based on their ability to act upon a detectable substrate. A compound or ligand can be assayed based on its ability to bind to a particular target molecule or molecules.

[0043] By a "set" of compounds is meant a collection of compounds. The compounds may or may not be structurally related.

[0044] In another aspect, structural information about PDE5A can also be used to assist in determining a struture for another phosphodiesterase, *e.g.*, a PDE2, by creating a homology model from an electronic representation of a PDE5A structure.

[0045] Typically creating such a homology model involves identifying conserved amino acid residues between PDE5A and the other phosphodiesterase of interest; transferring the atomic coordinates of a plurality of conserved amino acids in the PDE5A structure to the corresponding amino acids of the other phosphodiesterase to provide a rough structure of that phosphodiesterase; and constructing structures representing the remainder of the other phosphodiesterase using electronic representations of the structures of the remaining amino acid residues in the other phosphodiesterase. In particular, coordinates from Table 1 for conserved residues can be used. Conserved residues in a binding site can be used.

[0046] To assist in developing other portions of the phosphodiesterase structure, the homology model can also utilize, or be fitted with, low resolution x-ray diffraction data from one or more crystals of the phosphodiesterase, *e.g.*, to assist in linking conserved residues and/or to better specify coordinates for terminal portions of a polypeptide.

[0047] The PDE5A structural information used can be for a variety of different PDE5A variants, including full-length wild type, naturally-occurring variants (*e.g.*, allelic variants and splice variants), truncated variants of wild type or naturally-occuring variants, and mutants of full-length or truncated wild-type or naturally-occurring variants (that can be mutated at one or more sites). For example, in order to provide a PDE5A structure closer to a variety of other phosphodiesterase structures, a mutated PDE5A that includes a mutation to a conserved residue in a binding site can be used.

[0048] In another aspect, the invention provides a crystalline form of PDE5A, which may be a reduced length PDE5A such as a PDE5A phosphodiesterase domain, *e.g.*, having atomic coordinates as described in Table 1. The crystalline form can contain one or more heavy metal atoms, for example, atoms useful for X-ray crystallography. The crystalline form can also include a binding compound in a co-crystal, *e.g.*, a binding compound that interacts with one more more conserved PDE5A active site residues, or any two, any three, any four, any five, any six of those residues, and can, for example, be a known PDE5A inhibitor. PDE5A crystals can be in various environments, *e.g.*, in a crystallography plate, mounted for X-ray crystallography, and/or in an X-ray beam. The PDE5A may be of

various forms, *e.g.*, a wild-type, variant, truncated, and/or mutated form as described herein.

[0049] The invention further concerns co-crystals of PDE5A, which may be a reduced length PDE5A, *e.g.*, a PDE5A phosphodiesterase domain, and a PDE5A binding compound. Advantageously, such co-crystals are of sufficient size and quality to allow structural determination of PDE5A to at least 3 Angstroms, 2.5 Angstroms, 2.0 Angstroms, or 1.8 Angstroms. The co-crystals can, for example, be in a crystallography plate, be mounted for X-ray crystallography and/or in an X-ray beam. Such co-crystals are beneficial, for example, for obtaining structural information concerning interaction between PDE5A and binding compounds.

[0050] PDE5A binding compounds can include compounds that interact with at least one of conserved PDE5A active site residues, or any 2, 3, 4, 5, or 6 of those residues. Exemplary compounds that bind to PDE5A include compounds described in references cited herein.

[0051] Likewise, in additional aspects, methods for obtaining PDE5A crystals and co-crystals are provided. In one aspect is provided a method for obtaining a crystal of PDE5A phosphodiesterase domain, by subjecting PDE5A phosphodiesterase domain protein at 5-20 mg/ml, preferably 8-12 mg/ml, to crystallization condition substantially equivalent to: 10% (w/v) PEG3000, 100 mM phosphate-citrate (pH 4.3), 200 mM NaCl, 1mM DTT, 1mM Sp-cAMP. In general, the PDE5A will be in a solution containing the protein and suitable buffer.

[0052] Crystallization conditions can be initially identified using a screening kit, such as a Hampton Research (Riverside, CA) screening kit 1. Conditions resulting in crystals can be selected and crystallization conditions optimized based on the demonstrated crystallization conditions. To assist in subsequent crystallography, the PDE5A can be seleno-methionine labeled. Also, as indicated above, the PDE5A may be any of various forms, *e.g.*, truncated to provide a PDE5A phosphodiesterase domain, which can be selected to be of various lengths.

[0053] A related aspect provides a method for obtaining co-crystals of PDE5A, which can be a reduced length PDE5A, with a binding compound, by subjecting PDE5A protein

at 5-20 mg/ml to crystallization conditions substantially equivalent to10% (w/v) PEG3000, 100 mM phosphate-citrate (pH 4.3), 200 mM NaCl, 1mM DTT, 1mM Sp-cAMP, in the presence of binding compound, for a time sufficient for cystal development. The binding compound may be added at various concentrations depending on the nature of the comound, *e.g.*, final concentration of 0.5 to 1.0 mM. In many cases, the binding compound will be in an organic solvent such as demethyl sulfoxide solution (DMSO). While not preferred, binding compound can also be soaked into a PDE5A crystal, *e.g.*, using conventional techniques.

[0054] In another aspect, provision of compounds active on PDE5A also provides a method for modulating PDE5A activity by contacting PDE5A with a compound that binds to PDE5A and interacts with one more conserved PDE5A active site residues, where the compound has been identified using a PDE5A crystal structure. The compound is preferably provided at a level sufficient to modulate the activity of PDE5A by at least 10%, more preferably at least 20%, 30%, 40%, or 50%. In many embodiments, the compound will be at a concentration of about 1 μM, 100 μM, or 1 mM, or in a range of 1-100 nM, 100-500 nM, 500-1000 nM, 1-100 μM, 100-500 μM, or 500-1000 μM.

[0055] As used herein, the term "modulating" or "modulate" refers to an effect of altering a biological activity, especially a biological activity associated with a particular biomolecule such as PDE5A. For example, an agonist or antagonist of a particular biomolecule modulates the activity of that biomolecule, *e.g.*, an enzyme.

[0056] The term "PDE5A activity" refers to a biological activity of PDE5A, particularly including phosphodiesterase activity.

[0057] In the context of the use, testing, or screening of compounds that are or may be modulators, the term "contacting" means that the compound(s) are caused to be in sufficient proximity to a particular molecule, complex, cell, tissue, organism, or other specified material that potential binding interactions and/or chemical reaction between the compound and other specified material can occur.

[0058] In a related aspect, the invention provides a method for treating a patient suffering from a disease or condition characterized by abnormal PDE5A

phosphodiesterase activity, where the method involves administering to the patient a compound identified by fitting to a PDE5A crystal structure.

[0059] Specific diseases or disorders which might be treated or prevented include those described in the Detailed Description herein, and in the references cited therein.

[0060] As crystals of PDE5A have been developed and analyzed, another aspect concerns an electronic representation of PDE5A (which may be a reduced length PDE5A), for example, an electronic representation containing atomic coordinate representations corresponding to the coordinates listed for PDE5A in Table 1, or a schematic representation such as one showing secondary structure and/or chain folding, and may also show conserved active site residues. The PDE5A may be wild type, an allelic variant, a mutant form, or a modifed form, *e.g.*, as described herein.

[0061] The electronic representation can also be modified by replacing electronic representations of particular residues with electronic representations of other residues. Thus, for example, an electronic representation containing atomic coordinate representations corresponding to the coordinates for PDE5A listed in Table 1 can be modified by the replacement of coordinates for a particular conserved residue in a binding site by a different amino acid. Likewise, a PDE5A representation can be modified by the respective substitutions, insertions, and/or deletions of amino acid residues to provide a representation of a structure for PDE6 or PDE11. Following a modification or modifications, the representation of the overall structure can be adjusted to allow for the known interactions that would be affected by the modification or modifications. In most cases, a modification involving more than one residue will be performed in an iterative manner.

[0062] In addition, an electronic representation of a PDE5A binding compound or a test compound in the binding site can be included, *e.g.*, a non-hydrolyzable cGMP analog.

[0063] Likewise, in a related aspect, the invention concerns an electronic representation of a portion of PDE5A, a binding site (which can be an active site) or phosphodiesterase domain, for example, residues 531-875 or other phosphodiesterase domain described herein, such as the amino acid sequence provided in Table 2. A binding site or phosphodiesterase domain can be represented in various ways, *e.g.*, as representations of

atomic coordinates of residues around the binding site and/or as a binding site surface contour, and can include representations of the binding character of particular residues at the binding site, e.g., conserved residues. As for electronic representations of PDE5A, a binding compound or test compound may be present in the binding site; the binding site may be of a wild type, variant, mutant form, or modified form of PDE5A.

[0064] In yet another aspect, the structural information of PDE5A can be used in a homology model (based on PDE5A) for another phosphodiesterase (such as PDE6 or PDE11), thus providing an electronic representation of a PDE5A based homology model for a phosphodiesterase. For example, the homology model can utilize atomic coordinates from Table 1 for conserved amino acid residues. In particular embodiments; atomic coordinates for a wild type, variant, modified form, or mutated form of PDE5A can be used, including, for example, wild type, variants, modified forms, and mutant forms as described herein. In particular, PDE5A structure provides a very close homology model for PDE6 and PDE11. Thus, in particular embodiments the invention provides PDE5A-based homology models of PDE6 and PDE11.

[0065] In still another aspect, the invention provides an electronic representation of a modified PDE5A crystal structure, that includes an electronic representation of the atomic coordinates of a modified PDE5A. In an exemplary embodiment, atomic coordinates of Table 1 can be modified by the replacement of atomic coordinates for a conserved residue with atomic coordinates for a different amino acid. Modifications can include substitutions, deletions (e.g., C-terminal and/or N-terminal delections), insertions (internal, C-terminal, and/or N-terminal) and/or side chain modifications.

[0066] In another aspect, the PDE5A structural information provides a method for developing useful biological agents based on PDE5A, by analyzing a PDE5A structure to identify at least one sub-structure for forming the biological agent. Such sub-structures can include epitopes for antibody formation, and the method includes developing antibodies against the epitopes, *e.g.*, by injecting an epitope presenting composition in a mammal such as a rabbit, guinea pig, pig, goat, or horse. The sub-structure can also include a mutation site at which mutation is expected to or is known to alter the activity of the PDE5A, and the method includes creating a mutation at that site. Still further, the sub-structure can include an attachment point for attaching a separate moiety, for example, a

peptide, a polypeptide, a solid phase material (*e.g.*, beads, gels, chromatographic media, slides, chips, plates, and well surfaces), a linker, and a label (*e.g.*, a direct label such as a fluorophore or an indirect label, such as biotin or other member of a specific binding pair). The method can include attaching the separate moiety.

[0067] In another aspect, the invention provides a method for identifying potential PDE5A, binding compounds by fitting at least one electronic representation of a compound in an electronic representation of a PDE5A binding site. The representation of the binding site may be part of an electronic representation of a larger portion(s) or all of a PDE5A molecule or may be a representation of only the binding site or active site. The electronic representation may be as described above or otherwise described herein. For example, the compound may be a molecular scaffold, a derivative of a molecular scaffold, or a compound that is structurally similar to such molecular scaffold or derivative thereof.

[0068] In particular embodiments, the method involves fitting a computer representation of a compound from a computer database with a computer representation of the active site of PDE5A, and involves removing a computer representation of a compound complexed with the PDE5A molecule and identifying compounds that best fit the active site based on favorable geometric fit and energetically favorable complementary interactions as potential binding compounds. In particular embodiments, the compound is a known PDE5A inhibitor, *e.g.*, as described in a reference cited herein, or a derivative thereof.

[0069] In other embodiments, the method involves modifying a computer representation of a compound complexed with a PDE5A molecule, by the deletion or addition or both of one or more chemical groups; fitting a computer representation of a compound from a computer database with a computer representation of the active site of the PDE5A molecule; and identifying compounds that best fit the active site based on favorable geometric fit and energetically favorable complementary interactions as potential binding compounds.

[0070] In still other embodiments, the method involves removing a computer representation of a compound complexed with PDE5A, and searching a database for compounds having structural similarity to the complexed compound using a compound searching computer program or replacing portions of the complexed compound with similar chemical structures using a compound construction computer program.

[0071] Fitting a compound can include determining whether a compound will interact with one or more conserved PDE5A active site residues. Compounds selected for fitting or that are complexed with PDE5A can, for example, be a known PDE5A inhibitor compound.

[0072] In another aspect, the invention concerns a method for attaching a PDE5A binding compound to an attachment component, as well as a method for indentifying attachment sites on a PDE5A binding compound. The method involves identifying energetically allowed sites for attachment of an attachment component for the binding compound bound to a binding site of PDE5A; and attaching the compound or a derivative thereof to the attachment component at the energetically allowed site.

[0073] Attachment components can include, for example, linkers (including traceless linkers) for attachment to a solid phase or to another molecule or other moiety. Such attachment can be formed by synthesizing the compound or derivative on the linker attached to a solid phase medium e.g., in a combinatorial synthesis in a plurality of compound. Likewise, the attachment to a solid phase medium can provide an affinity medium (e.g., for affinity chromatography).

[0074] The attachment component can also include a label, which can be a directly detectable label such as a fluorophore, or an indirectly detectable such as a member of a specific binding pair, e.g., biotin.

[0075] The ability to identify energentically allowed sites on a PDE5A binding compound, also, in a related aspect, provides modified binding compounds that have linkers attached, preferably at an energetically allowed site for binding of the modified compound to PDE5A. The linker can be attached to an attachment component as described above.

[0076] Another aspect concerns a modified PDE5A polypeptide that includes a modification that makes the modified PDE5A more similar than native PDE5A to another phosphodiesterase, and can also include other mutations or other modifications. In various embodiments, the polypeptide includes a full-length PDE5A polypeptide, includes a modified PDE5A binding site, includes at least 20, 30, 40, 50, 60, 70, or 80 contiguous amino acid residues derived from PDE5A including a conserved site.

[0077] Still another aspect of the invention concerns a method for developing a ligand for a phosphodiesterase that includes conserved residues matching any one, 2, 3, 4, 5, or 6 of conserved PDE5A active site residues, by determining whether a compound binds to the phosphodiesterase and interacts with such active site residues in a PDE5A crystal. The method can also include determining whether the compound modulates the activity of the phosphodiesterase. Preferably the phosphodiesterase has at least 50, 55, 60, or 70% identity over an equal length phosphodiesterase domain segment.

[0078] In particular embodiments, the determining includes computer fitting the compound in a binding site of the phosphodiesterase and/or the method includes forming a co-crystal of the phosphodiesterase and the compound. Such co-crystals can be used for determing the binding orientation of the compound with the phosphodiesterase and/or provide structural information on the phosphodiesterase, e.g., on the binding site and interacting amino acid residues. Such binding orientation and/or other structural information can be accomplished using X-ray crystallography.

[0079] The invention also provides compounds that bind to and/or modulate (*e.g.*, inhibit) PDE5A, *e.g.*, PDE5A phosphodiesterase activity. Accordingly, in aspects and embodiments involving PDE5A binding compounds, molecular scaffolds, and ligands or modulators, the compound is a weak binding compound; a moderate binding compound; a strong binding compound; the compound interacts with one or more conserved PDE5A active site residues; the compound is a small molecule; the compound binds to a plurality of different phosphodiesterases (*e.g.*, at least 2, 3, 4, 5, 7, 10, or more different phosphodiesterases).

[0080] Additional aspects and embodiments will be apparent from the following Detailed Description and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0081] FIGURE 1 shows a ribbon diagram schematic representation of PDE5A phosphodiesterase domain having the sequence in Table 2.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0082] The Tables will first be briefly described.

[0083] Table 1 provides atomic coordinates for human PDE5A phosphodiesterase domain. In this table, the various columns have the following content, beginning with the left-most column:
ATOM: Refers to the relevant moeity for the table row.
Atom number: Refers to the arbitrary atom number designation within the coordinate table.
Atom Name: Identifier for the atom present at the particular coordinates.
Chain ID: Chain ID refers to one monomer of the protein in the crystal, *e.g.*, chain "A", or to other compound present in the crystal, e.g., HOH for water, and L for a ligand or binding compound. Multiple copies of the protein monomers will have different chain Ids.
Residue Number: The amino acid residue number in the chain.
X, Y, Z: Respectively are the X, Y, and Z coordinate values.
Occupancy: Describes the fraction of time the atom is observed in the crystal. For example, occupancy = 1 means that the atom is present all the time; occupancy = o.5 indicates that the atom is present in the location 50% of the time.
B-factor: A measure of the thermal motion of the atom.
Element: Identifier for the element.

[0084] Table 2 provides amino acid and nucleic acid sequences for a PDE5A phosphodiesterase domain. Numbering on the amino acid sequence does not correspond to standard numbering for native PDE5A.

[0085] Table 3 provides an alignment of phosphodiesterase domains for several phosphodiesterases, including human PDE5A, providing identification of residues conserved between various members of the set.

[0086] Table 4 provides the nucleic acid and amino acid sequences for human PDE5A phosphodiesterase.

I. General and PDE5 Inhibitors

[0087] The present invention concerns the use of PDE5A phosphodiesterase structures, structural information, and related compositions for identifying compounds that modulate PDE5A phosphodiesterase activity and for determining structuctures of other phosphodiesterases.

[0088] PDE5A is involved in a number of disease and conditions, and thus can be targeted in therapeutic and prophylactic methods.

[0089] A large number of compounds that are active on PDE5, from several different chemical classes, have been identified, and pharmaceutical products directed to PDE5 have been developed and approved by the Food and Drug Administration. Such compounds can be used in conjuction with crystal structure information on PDE5A to develop improved inhibitors.

[0090] The following are among the examples of descriptions of such compounds. The compounds described in the publications listed can be used in the present invention to develop improved PDE5 inhibitors, *e.g.*, inhibitors with improved affinity, activity, and/or specificity properties. Bunnage et al., U.S. Patent 6,333,330, U.S. Patent 6,407,114, and U.S. Patent Publication 2001/0039271, all entitled PYRAZOLOPYRIMIDINONE CGMP PDE5 INHIBITORS FOR THE TREATEMENT OF SEXUAL DYSFUNCTION, describe some pyrazolopyrimidinone compounds and their synthesis, preparation of pharmaceutical compositions, and administration. Fryburg et al., U.S. Patent Application Publication 2002/0165237, entitled TREATMENT OF THE INSULIN RESISTANCE SYNDROME, lists a variety of PDE5 inhibitors, including compounds described in EP-A-0463756, EP-A-0526004, WO 93/06104, 93/07149, WO 93/12095. WO 94/00453, WO 98/49166, WO 99/54333, EP-A-0995751, WO 00/24745, EP-A-995750, WO 95/19978, and WO 93/07124, along with methods for formulating and administering pharmaceutical compositions. Bombrun, U.S. Patent 6,043,252, entitled CARBOLINE DERIVATIVES, describes PDE5 inhibitors that are carboline derivatives. Allerton, U.S. Patent Application Publication 2002/0173502, entitled PHARMACEUTICALLY ACTIVE COMPOUNDS, describes as PDE5 inhibitors certain compounds that include four heterocyclic groups. Sperl et al., U.S. Patent 6,066,634 describes substituted condensation products of N-benzyl-3-indenylacetamides herocyclic aldehydes and their use in treatment of neoplasias. Additional PDE5 inhibitors are described in Maw, U.S. Patent 6,503,908; Maw et al., U.S.

Patent 6,440,982; Daugan et al., U.S. Patent 6,143,757; Daugan et al., U.S. Patent 6,143,746; Gonzalez et al., U.S. Patent Application Publication 2002/0058606. Benzimidazole derivatives with PDE5 inhibitor activity, and their preparation and use are described in Yamasaki et al., U.S. Patent 6,166,219. All of the above references are incorporated herein by reference in their entireties.

Exemplary Diseases Associated with PDE5A.

[0091] PDE5A has been correlated with several conditions in which inhibition of PDE5A is useful. Best known is the involvement of PDE5A in treatment of erectile dysfunction. Erection is largely a haemodynamic event that is regulated by fascular tone and blood-flow balance in the penis. Because cGMP levels modulate vascular tone, PDE5A is a useful target for intervention. When a man is sexually stimulated, nitric oxide (NO) is released from non-cholinergic, non-adrenergic neurons in the penis as well as from endothelial cells. NO diffuses into cells, where it activates soluble guanylyl cyclase, the enzyme that converts GTP to cGMP. The cGMP then stimulates PKG, which initiates a protein phosphorylation cascade. This results in a descrease in intracellular levels of cancium oins, leading ultimately to dilation of the arteries that bring blood to the penis and compression o the spongy corpus-cavernosum tissue. This compression contracts veins, which reduces the outflow of blood and increases intracavernosal pressure resulting in an erection. A PDE5A inhibitor retards enzymatic hydrolysis of cGMP in the corpus cavernosum, leading to the same outcome. (Rotella, 2002, Phosphodiesterase 5 inhibitors: Current status and potential applications, *Nature Reviews* 1:674-682.) (See also, Taher et al., *J. Urol.* 149:285A (1993); Murray, *DN&P* 6(3):150-156 (1993); Emmick et al., U.S. Patent 6,451,807, entitled METHODS OF TREATING SEXUAL DYSFUNCTION IN AN INDIVIDUAL SUFFEREING FROM A RETINAL DISEASE, CLASS 1 CONGESTIVE HEART FAILURE, OR MYOCARDIAL INFARCTION USING A PDE5 INHBITOR.)

[0092] In addition to treating erectile dysfunction, PDE inhibitors are described for use in treatment of premature ejaculation in individuals with normal erectile function. Boolell, U.S. Patent Application Publication 2002/0091129.

[0093] The use of PDE5A inhibitors in treatment of cystic fibrosis has also been indicated.

[0094] Treatment of Parkinson's Disease (PD) using PDE5 inhibitors has also been indicated. For example, Roylance, U.S. Patent 6,492,371, indicates that PDE5 inhibitors are useful in methods for preventing and/or slowing the progression of PD or reducing or eliminating clinical symptoms of PD.

[0095] Watkins et al., U.S. Patent Application Publication 2002/0128171 describes the use of PDE5 inhibitors to treat gastrointestinal disorders, such as disorders characterized by hypomobility or hypermobility of small intesting, large intestine, colon, esphagus, or stomach.

[0096] The vasodilatory effects of PDE5A inhibitors allows their use in connection with some circulatory disorders. In conjunction with a prostaglandin analogue (e.g., iloprost), a PDE5A inhibitor can enhance reduction of pulmonary arterial pressure, allowing such use in patients with pulmonary hypertension.

[0097] Subarachnoid haemorrhage is a significant cause of stroke in many patients. It often occurs as a consequence of reduced responsiveness to NO in cerebral srteries. To counter this effect, PDE5A inhibitors can elevate cellular levels of cGMP in cerbral arteries, thereby at least partially correcting the vascular dysfunction.

[0098] Shahinpoor et al., U.S. Patent Application Publication 2002/0168424 describes the use of PDE5 inhibitors in conjunction with a nitric oxid donor for treatment of glaucoma. The publication indicates the drugs work synergistically to reduce intraocular pressure.

[0099] PDE5A inhibitors also moderate platelet aggregation in a dose-dependent manner.

[0100] Fryburg et al., U.S. Patent Application Publication 2002/0165,237, entitled TREATEMENT OF THE INSULIN RESISTANCE SYNDROME, describes the use of selective PDE5 inhibitors in the curative, palliative, or prophylactic treatment of insulin resistance syndrome (also referred to as Syndrome X and Metabolic Syndrome). Insulin resistance syndrome means the concomitant existence of two or more of: dyslipidemia, hypertension, type 2 diabetes mellitus or a family history of type 2 diabetes mellitus, hyperuricaemia, and/or gout, a pro-coagulant state, atheroslerosis, truncal obesity.

[0101] Thompson et al., U.S. Patent 6,130,053, entitled METHODS FOR SELECTING COMPOUNDS FOR INHIBITON OF NEOPLASTIC LESIONS, and Thompson et al., U.S. Patent Application Publication 2002/0009764, entitled METHODS FOR IDENTIFYING COMPOUNDS FOR INHIBITON OF NEOPLASTIC LESIONS, AND PHARMACEUTICAL COMPOSITIONS CONTAINING SUCH COMPOUNDS describes the use of PDE5 inhibitors in conjunction with inhibition of PDE2 activity, leading to cell apoptosis, and methods for identifying useful compounds. See also, Pamakcu et al., U.S. Patent 6,500,610, entitled METHODS FOR IDENTIFYING COMPOUNDS FOR INHIBITING NEOPLASTIC LESIONS, AND PHARMACEUTICAL COMPOSITIONS CONTAINING SUCH COMPOUNDS. Similarly, Whitehead, U.S. Patent 6,479,493 describes the use of PDE2 inhibition combined with PDE5 inhibition for treatment of Type 1 diabetes, and describes compouns fo that purpose. Use of combination PDE2 and PDE5 inhibition is also described in Earle et al., U.S. Patent 6,465,494, entitled METHODS FOR TREATMENT OF CYSTIC FIBROSIS.

[0102] Bombrun, U.S. Patent 6,043,252 indicates that PDE5 inhibitors are useful for treatment of stable, unstable, and variant (Prinzmetal) angina, hypertension, pulmonary hypertenion, chronic obstructive pulmonary disease, congestive heart failure, acute respiratory distress syndrome, acute and chronic renal failure, atherosclerosis, conditions of reduced blood vessel patency (e.g., post-PTCA or post-bypass graft stenosis), peripheral vascular disease, vascular disorders such as Raynaud's disease, myocardial infarction, prophylaxis of stroke, stroke, bronchitis, chronic asthma, allergic asthma, allergic rhinitis, hypertrophy, , male and female erictile dysfunction, and diseases characterized by disorders of gut motility.

[0103] Davies et al., U.S. Patent Application Publication 2002/0065286 describes the use of PDE5 inhibitors in wound treatment, such chronic wounds of non-diabetic origin, as well as acute wounds, such as in the elderly.

[0104] The present methods can be used for developing ligands for treating one or more of the diseases and conditions above, or for other diseases or conditions for which PDE5A modulation is found useful.

II. Crystalline PDE5A

[0105] Crystalline PDE5A (*e.g.*, human PDE5A) include native crystals, phosphodiesterase domain crystals, derivative crystals and co-crystals. The native crystals generally comprise substantially pure polypeptides corresponding to PDE5A in crystalline form. PDE5A phosphodiesterase domain crystals generally comprise substantially pure PDE5A phosphodiesterase domain in crystalline form. In connection with the development of inhibitors of PDE5A phosphodiesterase function, it is advantageous to use PDE5A phosphodiesterase domain for structural determination, because use of the reduced sequence simplifies structure determination. To be useful for this purpose, the phosphodiesterase domain should be active and/or retain native-type binding, thus indicating that the phosphodiesterase domain takes on substantially normal 3D structure.

[0106] It is to be understood that the crystalline phosphodiesterases and phosphodiesterase domains of the invention are not limited to naturally occurring or native phosphodiesterase. Indeed, the crystals of the invention include crystals of mutants of native phosphodiesterases. Mutants of native phosphodiesterases are obtained by replacing at least one amino acid residue in a native phosphodiesterase with a different amino acid residue, or by adding or deleting amino acid residues within the native polypeptide or at the N- or C-terminus of the native polypeptide, and have substantially the same three-dimensional structure as the native phosphodiesterase from which the mutant is derived.

[0107] By having substantially the same three-dimensional structure is meant having a set of atomic structure coordinates that have a root-mean-square deviation of less than or equal to about 2Å when superimposed with the atomic structure coordinates of the native phosphodiesterase from which the mutant is derived when at least about 50% to 100% of the Cα atoms of the native phosphodiesterase domain are included in the superposition.

[0108] Amino acid substitutions, deletions and additions which do not significantly interfere with the three-dimensional structure of the phosphodiesterase will depend, in part, on the region of the phosphodiesterase where the substitution, addition or deletion occurs. In highly variable regions of the molecule, non-conservative substitutions as well as conservative substitutions may be tolerated without significantly disrupting the three-dimensional, structure of the molecule. In highly conserved regions, or regions containing significant secondary structure, conservative amino acid substitutions are preferred. Such

conserved and variable regions can be identified by sequence alignment of PDE5A with other phosphodiesterases. Such alignment of PDE5A phosphodiesterase domain along with a number of other phosphodiesterase domains is provided in Table 3.

[0109] Conservative amino acid substitutions are well known in the art, and include substitutions made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the amino acid residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine. Other conservative amino acid substitutions are well known in the art.

[0110] For phosphodiesterases obtained in whole or in part by chemical synthesis, the selection of amino acids available for substitution or addition is not limited to the genetically encoded amino acids. Indeed, the mutants described herein may contain non-genetically encoded amino acids. Conservative amino acid substitutions for many of the commonly known non-genetically encoded amino acids are well known in the art. Conservative substitutions for other amino acids can be determined based on their physical properties as compared to the properties of the genetically encoded amino acids.

[0111] In some instances, it may be particularly advantageous or convenient to substitute, delete and/or add amino acid residues to a native phosphodiesterase in order to provide convenient cloning sites in cDNA encoding the polypeptide, to aid in purification of the polypeptide, and for crystallization of the polypeptide. Such substitutions, deletions and/or additions which do not substantially alter the three dimensional structure of the native phosphodiesterase domain will be apparent to those of ordinary skill in the art.

[0112] It should be noted that the mutants contemplated herein need not all exhibit phosphodiesterase activity. Indeed, amino acid substitutions, additions or deletions that interfere with the phosphodiesterase activity but which do not significantly alter the three-dimensional structure of the domain are specifically contemplated by the invention. Such crystalline polypeptides, or the atomic structure coordinates obtained therefrom, can be

used to identify compounds that bind to the native domain. These compounds can affect the activity of the native domain.

[0113] The derivative crystals of the invention can comprise a crystalline phosphodiesterase polypeptide in covalent association with one or more heavy metal atoms. The polypeptide may correspond to a native or a mutated phosphodiesterase. Heavy metal atoms useful for providing derivative crystals include, by way of example and not limitation, gold, mercury, selenium, etc.

[0114] The co-crystals of the invention generally comprise a crystalline phosphodiesterase domain polypeptide in association with one or more compounds. The association may be covalent or non-covalent. Such compounds include, but are not limited to, cofactors, substrates, substrate analogues, inhibitors, allosteric effectors, etc.

[0115] Exemplary mutations for PDE5A family phosphodiesterases include mutations making the phosphodiesterase active site more like the active site of PDE6 or PDE11. Such insertion is useful, for example, to assist in using PDE5A to model PDE6 or PDE11. Mutations at other sites can likewise be carried out, *e.g.*, to make a mutated PDE5A more similar to another phosphodiesterase for structure modeling and/or compound fitting purposes, such as a phosphodiesterase in the phosphodiesterase domain alignment in Table 3.

[0116] In addition to the PDE5A crystal structure described herein, a crystal-based structure of PDE5A catalytic domain is described in Brown et al., PCT Application PCT/IB02/04426, International Publication WO 03/038080. That structure (and associated atomic coordinate sets), as well as other structures and atomic coordinate sets that may be obtained can also be used as described herein.

III. Three Dimensional Structure Determination Using X-ray Crystallography

[0117] X-ray crystallography is a method of solving the three dimensional structures of molecules. The structure of a molecule is calculated from X-ray diffraction patterns using a crystal as a diffraction grating. Three dimensional structures of protein molecules arise from crystals grown from a concentrated aqueous solution of that protein. The process of X-ray crystallography can include the following steps:

(a) synthesizing and isolating (or otherwise obtaining) a polypeptide;

(b) growing a crystal from an aqueous solution comprising the polypeptide with or without a modulator; and

(c) collecting X-ray diffraction patterns from the crystals, determining unit cell dimensions and symmetry, determining electron density, fitting the amino acid sequence of the polypeptide to the electron density, and refining the structure.

Production of Polypeptides

[0118] The native and mutated phosphodiesterase polypeptides described herein may be chemically synthesized in whole or part using techniques that are well-known in the art (*see, e.g.*, Creighton (1983) *Biopolymers* 22(1):49-58).

[0119] Alternatively, methods which are well known to those skilled in the art can be used to construct expression vectors containing the native or mutated phosphodiesterase polypeptide coding sequence and appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Maniatis, T (1989). Molecular cloning: A laboratory Manual. Cold Spring Harbor Laboratory, New York. Cold Spring Harbor Laboratory Press; and Ausubel, F.M. et al. (1994) Current Protocols in Molecular Biology. John Wiley & Sons, Secaucus, N.J.

[0120] A variety of host-expression vector systems may be utilized to express the phosphodiesterase coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the phosphodiesterase domain coding sequence; yeast transformed with recombinant yeast expression vectors containing the phosphodiesterase domain coding sequence; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the phosphodiesterase domain coding sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing the phosphodiesterase domain coding sequence; or animal cell systems. The expression elements of these systems vary in their strength and specificities.

[0121] Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used in the expression vector. For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage λ, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used; when cloning in insect cell systems, promoters such as the baculovirus polyhedrin promoter may be used; when cloning in plant cell systems, promoters derived from the genome of plant cells (*e.g.*, heat shock promoters; the promoter for the small subunit of RUBISCO; the promoter for the chlorophyll a/b binding protein) or from plant viruses (*e.g.*, the 35S RNA promoter of CaMV; the coat protein promoter of TMV) may be used; when cloning in mammalian cell systems, promoters derived from the genorne of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used; when generating cell lines that contain multiple copies of the phosphodiesterase domain DNA, SV4O-, BPV- and EBV-based vectors may be used with an appropriate selectable marker.

[0122] Exemplary methods describing methods of DNA manipulation, vectors, various types of cells used, methods of incorporating the vectors into the cells, expression techniques, protein purification and isolation methods, and protein concentration methods are disclosed in detail in PCT publication WO 96/18738. This publication is incorporated herein by reference in its entirety, including any drawings. Those skilled in the art will appreciate that such descriptions are applicable to the present invention and can be easily adapted to it.

Crystal Growth

[0123] Crystals are grown from an aqueous solution containing the purified and concentrated polypeptide by a variety of techniques. These techniques include batch, liquid, bridge, dialysis, vapor diffusion, and hanging drop methods. McPherson (1982) John Wiley, New York; McPherson (1990) *Eur. J. Biochem.* 189:1-23; Webber (1991) *Adv. Protein Chem.* 41:1-36, incorporated by reference herein in their entireties, including all figures, tables, and drawings.

[0124] The native crystals of the invention are, in general, grown by adding precipitants to the concentrated solution of the polypeptide. The precipitants are added at a

concentration just below that necessary to precipitate the protein. Water is removed by controlled evaporation to produce precipitating conditions, which are maintained until crystal growth ceases.

[0125] For crystals of the invention, exemplary crystallization conditions are described in the Examples. Those of ordinary skill in the art will recognize that the exemplary crystallization conditions can be varied. Such variations may be used alone or in combination. In addition, other crystallization conditions may be found, *e.g.*, by using crystallization screening plates to identify such other conditions. Those alternate conditions can then be optimized if needed to provide larger or better quality crystals.

[0126] Derivative crystals of the invention can be obtained by soaking native crystals in mother liquor containing salts of heavy metal atoms. It has been found that soaking a native crystal in a solution containing about 0.1 mM to about 5 mM thimerosal, 4-chloromeruribenzoic acid or $KAu(CN)_2$ for about 2 hr to about 72 hr provides derivative crystals suitable for use as isomorphous replacements in determining the X-ray crystal structure of PDE5A.

[0127] Co-crystals of the invention can be obtained by soaking a native crystal in mother liquor containing compound that binds the phosphodiesterase, or can be obtained by co-crystallizing the phosphodiesterase polypeptide in the presence of a binding compound.

[0128] Generally, co-crystallization of phosphodiesterase and binding compound can be accomplished using conditions identified for crystallizing the corresponding phosphodiesterase without binding compound. It is advantageous if a plurality of different crystallization conditions have been identified for the phosphodiesterase, and these can be tested to determine which condition gives the best co-crystals. It may also be benficial to optimize the conditions for co-crystallization. Alternatively, new crystallization conditions can be determined for obtaining co-crystals, *e.g.*, by screening for crystallization and then optimizing those conditions. Exemplary co-crystallization conditions are provided in the Examples.

Determining Unit Cell Dimensions and the Three Dimensional Structure of a Polypeptide or Polypeptide Complex

[0129] Once the crystal is grown, it can be placed in a glass capillary tube or other mounting device and mounted onto a holding device connected to an X-ray generator and an X-ray detection device. Collection of X-ray diffraction patterns are well documented by those in the art. See, *e.g.*, Ducruix and Geige, (1992), IRL Press, Oxford, England, and references cited therein. A beam of X-rays enters the crystal and then diffracts from the crystal. An X-ray detection device can be utilized to record the diffraction patterns emanating from the crystal. Although the X-ray detection device on older models of these instruments is a piece of film, modern instruments digitally record X-ray diffraction scattering. X-ray sources can be of various types, but advantageously, a high intensity source is used, *e.g.*, a synchrotron beam source.

[0130] Methods for obtaining the three dimensional structure of the crystalline form of a peptide molecule or molecule complex are well known in the art. See, *e.g.*, Ducruix and Geige, (1992), IRL Press, Oxford, England, and references cited therein. The following are steps in the process of determining the three dimensional structure of a molecule or complex from X-ray diffraction data.

[0131] After the X-ray diffraction patterns are collected from the crystal, the unit cell dimensions and orientation in the crystal can be determined. They can be determined from the spacing between the diffraction emissions as well as the patterns made from these emissions. The unit cell dimensions are characterized in three dimensions in units of Angstroms (one Å= 10^{-10} meters) and by angles at each vertices. The symmetry of the unit cell in the crystals is also characterized at this stage. The symmetry of the unit cell in the crystal simplifies the complexity of the collected data by identifying repeating patterns. Application of the symmetry and dimensions of the unit cell is described below.

[0132] Each diffraction pattern emission is characterized as a vector and the data collected at this stage of the method determines the amplitude of each vector. The phases of the vectors can be determined using multiple techniques. In one method, heavy atoms can be soaked into a crystal, a method called isomorphous replacement, and the phases of the vectors can be determined by using these heavy atoms as reference points in the X-ray analysis. (Otwinowski, (1991), Daresbury, United Kingdom, 80-86). The isomorphous replacement method usually utilizes more than one heavy atom derivative.

[0133] In another method, the amplitudes and phases of vectors from a crystalline polypeptide with an already determined structure can be applied to the amplitudes of the vectors from a crystalline polypeptide of unknown structure and consequently determine the phases of these vectors. This second method is known as molecular replacement and the protein structure which is used as a reference must have a closely related structure to the protein of interest. (Naraza (1994) *Proteins* 11:281-296). Thus, the vector information from a phosphodiesterase of known structure, such as those reported herein, are useful for the molecular replacement analysis of another phosphodiesterase with unknown structure.

[0134] Once the phases of the vectors describing the unit cell of a crystal are determined, the vector amplitudes and phases, unit cell dimensions, and unit cell symmetry can be used as terms in a Fourier transform function. The Fourier transform function calculates the electron density in the unit cell from these measurements. The electron density that describes one of the molecules or one of the molecule complexes in the unit cell can be referred to as an electron density map. The amino acid structures of the sequence or the molecular structures of compounds complexed with the crystalline polypeptide may then be fitted to the electron density using a variety of computer programs. This step of the process is sometimes referred to as model building and can be accomplished by using computer programs such as Turbo/FRODO or "O". (Jones (1985) *Methods in Enzymology* 115:157-171).

[0135] A theoretical electron density map can then be calculated from the amino acid structures fit to the experimentally determined electron density. The theoretical and experimental electron density maps can be compared to one another and the agreement between these two maps can be described by a parameter called an R-factor. A low value for an R-factor describes a high degree of overlapping electron density between a theoretical and experimental electron density map.

[0136] The R-factor is then minimized by using computer programs that refine the theoretical electron density map. A computer program such as X-PLOR can be used for model refinement by those skilled in the art. (Brünger (1992) *Nature* 355:472-475.) Refinement may be achieved in an iterative process. A first step can entail altering the conformation of atoms defined in an electron density map. The conformations of the

atoms can be altered by simulating a rise in temperature, which will increase the vibrational frequency of the bonds and modify positions of atoms in the structure. At a particular point in the atomic perturbation process, a force field, which typically defines interactions between atoms in terms of allowed bond angles and bond lengths, Van der Waals interactions, hydrogen bonds, ionic interactions, and hydrophobic interactions, can be applied to the system of atoms. Favorable interactions may be described in terms of free energy and the atoms can be moved over many iterations until a free energy minimum is achieved. The refinement process can be iterated until the R-factor reaches a minimum value.

[0137] The three dimensional structure of the molecule or molecule complex is described by atoms that fit the theoretical electron density characterized by a minimum R-value. A file can then be created for the three dimensional structure that defines each atom by coordinates in three dimensions. An example of such a structural coordinate file is shown in Table 1.

IV. Structures of PDE5A

[0138] The present invention provides high-resolution three-dimensional structures and atomic structure coordinates of crystalline PDE5A phosphodiesterase domain and PDE5A phosphodiesterase domain co-complexed with exemplary binding compounds as determined by X-ray crystallography. The methods used to obtain the structure coordinates are provided in the examples. The atomic structure coordinates of crystalline PDE5A are listed in Table 1. Co-crystal coordinates can be used in the same way, *e.g.*, in the various aspects described herein, as coordinates for the protein by itself.

[0139] Those having skill in the art will recognize that atomic structure coordinates as determined by X-ray crystallography are not without error. Thus, it is to be understood that any set of structure coordinates obtained for crystals of PDE5A, whether native crystals, phosphodiesterase domain crystals, derivative crystals or co-crystals, that have a root mean square deviation ("r.m.s.d.") of less than or equal to about 1.5 Å when superimposed, using backbone atoms (N, C_α, C and 0), on the structure coordinates listed in Table 1 are considered to be identical with the structure coordinates listed in the Table 1 when at least about 50% to 100% of the backbone atoms of PDE5A are included in the superposition.

[0140] As indicated above, a crystal-based PDE5A catalytic domain structure is described in Brown et al., PCT Application PCT/IB02/04426, International Publication WO 03/038080.

V. Uses of the Crystals and Atomic Structure Coordinates

[0141] The crystals of the invention, and particularly the atomic structure coordinates obtained therefrom, have a wide variety of uses. For example, the crystals described herein can be used as a starting point in any of the methods of use for phosphodiesterases known in the art or later developed. Such methods of use include, for example, identifying molecules that bind to the native or mutated catalytic domain of phosphodiesterases. The crystals and structure coordinates are particularly useful for identifying ligands that modulate phosphodiesterase activity as an approach towards developing new therapeutic agents. In particular, the crystals and structural information are useful in methods for ligand development utilizing molecular scaffolds.

[0142] The structure coordinates described herein can be used as phasing models for determining the crystal structures of additional phosphodiesterases, as well as the structures of co-crystals of such phosphodiesterases with ligands such as inhibitors, agonists, antagonists, and other molecules. The structure coordinates, as well as models of the three-dimensional structures obtained therefrom, can also be used to aid the elucidation of solution-based structures of native or mutated phosphodiesterases, such as those obtained via NMR.

VI. Electronic Representations of Phosphodiesterase Structures

[0143] Structural information of phosphodiesterases or portions of phosphodiesterases (*e.g.*, phosphodiesterase active sites) can be represented in many different ways. Particularly useful are electronic representations, as such representations allow rapid and convenient data manipulations and structural modifications. Electronic representations can be embedded in manydifferent storage or memory media, frequently computer readable media. Examples include without limitations, computer random access memory (RAM), floppy disk, magnetic hard drive, magnetic tape (analog or digital), compact disk (CD), optical disk, CD-ROM, memory card, digital video disk (DVD), and others. The storage medium can be separate or part of a computer system. Such a computer system may be a

dedicated, special purpose, or embedded system, such as a computer system that forms part of an X-ray crystallography system, or may be a general purpose computer (which may have data connection with other equipment such as a sensor device in an X-ray crystallographic system. In many cases, the information provided by such electronic representations can also be represented physically or visually in two or three dimensions, *e.g.*, on paper, as a visual display (*e.g.*, on a computer monitor as a two dimensional or pseudo-three dimensional image) or as a three dimensional physical model. Such physical representations can also be used, alone or in connection with electronic representations. Exemplary useful representations include, but are not limited to, the following:

Atomic Coordinate Representation

[0144] One type of representation is a list or table of atomic coordinates representing positions of particular atoms in a molecular structure, portions of a structure, or complex (*e.g.*, a co-crystal). Such a representation may also include additional information, for example, information about occupancy of particular coordinates. One such atomic coordinate representation contains the coordinate information of Table 1 in electronic form.

Energy Surface or Surface of Interaction Representation

[0145] Another representation is an energy surface representation, *e.g.*, of an active site or other binding site, representing an energy surface for electronic and steric interactions. Such a representation may also include other features. An example is the inclusion of representation of a particular amino acid residue(s) or group(s) on a particular amino acid residue(s), *e.g.*, a residue or group that can participate in H-bonding or ionic interaction. Such energy surface representations can be readily generated from atomic coordinate representations using any of a variety of available computer programs.

Structural Representation

[0146] Still another representation is a structural representation, *i.e.*, a physical representation or an electronic representation of such a physical representation. Such a structural representation includes representations of relative positions of particular features of a molecule or complex, often with linkage between structural features. For example, a structure can be represented in which all atoms are linked; atoms other than hydrogen are linked; backbone atoms, with or without representation of sidechain atoms that could

participate in significant electronic interaction, are linked; among others. However, not all features need to be linked. For example, for structural representations of portions of a molecule or complex, structural features significant for that feature may be represented (*e.g.*, atoms of amino acid residues that can have significant binding interation with a ligand at a binding site. Those amino acid residues may not be linked with each other.

[0147] A structural representation can also be a schematic representation. For example, a schematic representation can represent secondary and/or tertiary structure in a schematic manner. Within such a schematic representation of a polypeptide, a particular amino acid residue(s) or group(s) on a residue(s) can be included, *e.g.*, conserved residues in a binding site, and/or residue(s) or group(s) that may interact with binding compounds. Electronic structural representations can be generated, for example, from atomic coordinate information using computer programs designed for that function and/or by constructing an electronic representation with manual input based on interpretation of another form of structural information. Physical representations can be created, for example, by printing an image of a computer-generated image or by constructing a 3D model. An example of such a printed representation is the ribbon diagram presented in Figure 1.

VII. Structure Determination for Phosphodiesterases with Unknown Structure Using Structural Coordinates

[0148] Structural coordinates, such as those set forth in Table 1, can be used to determine the three dimensional structures of phosphodiesterases with unknown structure. The methods described below can apply structural coordinates of a polypeptide with known structure to another data set, such as an amino acid sequence, X-ray crystallographic diffraction data, or nuclear magnetic resonance (NMR) data. Preferred embodiments of the invention relate to determining the three dimensional structures of other PDE5A phosphodiesterases, other phosphodiesterases, and related polypeptides.

Structures Using Amino Acid Homology

[0149] Homology modeling is a method of applying structural coordinates of a polypeptide of known structure to the amino acid sequence of a polypeptide of unknown structure. This method is accomplished using a computer representation of the three dimensional structure of a polypeptide or polypeptide complex, the computer representation of amino acid sequences of the polypeptides with known and unknown

structures, and standard computer representations of the structures of amino acids. Homology modeling generally involves (a) aligning the amino acid sequences of the polypeptides with and without known structure; (b) transferring the coordinates of the conserved amino acids in the known structure to the corresponding amino acids of the polypeptide of unknown structure; refining the subsequent three dimensional structure; and (d) constructing structures of the rest of the polypeptide. One skilled in the art recognizes that conserved amino acids between two proteins can be determined from the sequence alignment step in step (a).

[0150] The above method is well known to those skilled in the art. (Greer (1985) *Science* 228:1055; Blundell et *al.* A(1988) *Eur. J. Biochem.* 172:513. An exemplary computer program that can be utilized for homology modeling by those skilled in the art is the Homology module in the Insight II modeling package distributed by Accelerys Inc.

[0151] Alignment of the amino acid sequence is accomplished by first placing the computer representation of the amino acid sequence of a polypeptide with known structure above the amino acid sequence of the polypeptide of unknown structure. Amino acids in the sequences are then compared and groups of amino acids that are homologous (e.g., amino acid side chains that are similar in chemical nature - aliphatic, aromatic, polar, or charged) are grouped together. This method will detect conserved regions of the polypeptides and account for amino acid insertions or deletions. Such alignment and/or can also be performed fully electronically using sequence alignment and analyses software.

[0152] Once the amino acid sequences of the polypeptides with known and unknown structures are aligned, the structures of the conserved amino acids in the computer representation of the polypeptide with known structure are transferred to the corresponding amino acids of the polypeptide whose structure is unknown. For example, a tyrosine in the amino acid sequence of known structure may be replaced by a phenylalanine, the corresponding homologous amino acid in the amino acid sequence of unknown structure.

[0153] The structures of amino acids located in non-conserved regions are to be assigned manually by either using standard peptide geometries or molecular simulation techniques, such as molecular dynamics. The final step in the process is accomplished by refining the

entire structure using molecular dynamics and/or energy minimization. The homology modeling method is well known to those skilled in the art and has been practiced using different protein molecules. For example, the three dimensional structure of the polypeptide corresponding to the catalytic domain of a serine/threonine protein kinase, myosin light chain protein kinase, was homology modeled from the cAMP-dependent protein kinase catalytic subunit. (Knighton et *al.* (1992) *Science* 258:130-135.)

Structures Using Molecular Replacement

[0154] Molecular replacement is a method of applying the X-ray diffraction data of a polypeptide of known structure to the X-ray diffraction data of a polypeptide of unknown sequence. This method can be utilized to define the phases describing the X-ray diffraction data of a polypeptide of unknown structure when only the amplitudes are known. X-PLOR is a commonly utilized computer software package used for molecular replacement. Brünger (1992) *Nature* 355:472-475. AMORE is another program used for molecular replacement. Navaza (1994) *Acta Crystallogr.* A50:157-163. Preferably, the resulting structure does not exhibit a root-mean-square deviation of more than 3Å.

[0155] A goal of molecular replacement is to align the positions of atoms in the unit cell by matching electron diffraction data from two crystals. A program such as X-PLOR can involve four steps. A first step can be to determine the number of molecules in the unit cell and define the angles between them. A second step can involve rotating the diffraction data to define the orientation of the molecules in the unit cell. A third step can be to translate the electron density in three dimensions to correctly position the molecules in the unit cell. Once the amplitudes and phases of the X-ray diffraction data is determined, an R-factor can be calculated by comparing electron diffraction maps calculated experimentally from the reference data set and calculated from the new data set. An R-factor between 30-50% indicates that the orientations of the atoms in the unit cell are reasonably determined by this method. A fourth step in the process can be to decrease the R-factor to roughly 20% by refining the new electron density map using iterative refinement techniques described herein and known to those or ordinary skill in the art.

Structures Using NMR Data

[0156] Structural coordinates of a polypeptide or polypeptide complex derived from X-ray crystallographic techniques can be applied towards the elucidation of three

dimensional structures of polypeptides from nuclear magnetic resonance (NMR) data. This method is used by those skilled in the art. (Wuthrich, (1986), John Wiley and Sons, New York:176-199; Pflugrath et *al.* (1986) *J. Mol. Biol.* 189:383-386; Kline *et al.* (1986) *J. Mol. Biol.* 189:377-382.) While the secondary structure of a polypeptide is often readily determined by utilizing two-dimensional NMR data, the spatial connections between individual pieces of secondary structure are not as readily determinable. The coordinates defining a three-dimensional structure of a polypeptide derived from X-ray crystallographic techniques can guide the NMR spectroscopist to an understanding of these spatial interactions between secondary structural elements in a polypeptide of related structure.

[0157] The knowledge of spatial interactions between secondary structural elements can greatly simplify Nuclear Overhauser Effect (NOE) data from two-dimensional NMR experiments. Additionally, applying the crystallographic coordinates after the determination of secondary structure by NMR techniques only simplifies the assignment of NOEs relating to particular amino acids in the polypeptide sequence and does not greatly bias the NMR analysis of polypeptide structure. Conversely, using the crystallographic coordinates to simplify NOE data while determining secondary structure of the polypeptide would bias the NMR analysis of protein structure.

VIII. Structure-Based Design of Modulators of Phosphodiesterase Function Utilizing Structural Coordinates

[0158] Structure-based modulator design and identification methods are powerful techniques that can involve searches of computer databases containing a wide variety of potential modulators and chemical functional groups. The computerized design and identification of modulators is useful as the computer databases contain more compounds than the chemical libraries, often by an order of magnitude. For reviews of structure-based drug design and identification (*see* Kuntz et *al.* (1994), *Acc. Chem. Res.* 27:117; Guida (1994) *Current Opinion in Struc. Biol.* 4: 777; Colman (1994) *Current Opinion in Struc. Biol.* 4: 868).

[0159] The three dimensional structure of a polypeptide defined by structural coordinates can be utilized by these design methods, for example, the structural coordinates of Table 1. In addition, the three dimensional structures of phosphodiesterases

determined by the homology, molecular replacement, and NMR techniques described herein can also be applied to modulator design and identification methods.

[0160] For identifying modulators, structural information for a native phosphodiesterase, in particular, structural information for the active site of the phosphodiesterase, can be used. However, it may be advantageous to utilize structural information from one or more co-crystals of the phosphodiesterase with one or more binding compounds. It can also be advantageous if the binding compound has a structural core in common with test compounds.

Design by Searching Molecular Data Bases

[0161] One method of rational design searches for modulators by docking the computer representations of compounds from a database of molecules. Publicly available databases include, for example:

a) ACD from Molecular Designs Limited
b) NCI from National Cancer Institute
c) CCDC from Cambridge Crystallographic Data Center
d) CAST from Chemical Abstract Service
e) Derwent from Derwent Information Limited
f) Maybridge from Maybridge Chemical Company LTD
g) Aldrich from Aldrich Chemical Company
h) Directory of Natural Products from Chapman & Hall

[0162] One such data base (ACD distributed by Molecular Designs Limited Information Systems) contains compounds that are synthetically derived or are natural products. Methods available to those skilled in the art can convert a data set represented in two dimensions to one represented in three dimensions. These methods are enabled by such computer programs as CONCORD from Tripos Associates or DE-Converter from Molecular Simulations Limited.

[0163] Multiple methods of structure-based modulator design are known to those in the art. (Kuntz et *al.*, (1982), *J. Mol. Biol. 162:* 269; Kuntz et aZ., (1994), Acc. *Chern. Res. 27:* 117; Meng *et al.*, (1992), *J. Compt. Chem. 13:* 505; Bohm, (1994), *J. Comp. Aided Molec. Design 8:* 623.)

[0164] A computer program widely utilized by those skilled in the art of rational modulator design is DOCK from the University of California in San Francisco. The general methods utilized by this computer program and programs like it are described in three applications below. More detailed information regarding some of these techniques can be found in the Accelerys User Guide, 1995. A typical computer program used for this purpose can perform a processes comprising the following steps or functions:

(a) remove the existing compound from the protein;

(b) dock the structure of another compound into the active-site using the computer program (such as DOCK) or by interactively moving the compound into the active-site;

(c) characterize the space between the compound and the active-site atoms;

(d) search libraries for molecular fragments which (i) can fit into the empty space between the compound and the active-site, and (ii) can be linked to the compound; and

(e) link the fragments found above to the compound and evaluate the new modified compound.

[0165] Part (c) refers to characterizing the geometry and the complementary interactions formed between the atoms of the active site and the compounds. A favorable geometric fit is attained when a significant surface area is shared between the compound and active-site atoms without forming unfavorable steric interactions.One skilled in the art would note that the method can be performed by skipping parts (d) and (e) and screening a database of many compounds.

[0166] Structure-based design and identification of modulators of phosphodiesterase function can be used in conjunction with assay screening. As large computer databases of compounds (around 10,000 compounds) can be searched in a matter of hours or even less, the computer-based method can narrow the compounds tested as potential modulators of phosphodiesterase function in biochemical or cellular assays.

[0167] The above descriptions of structure-based modulator design are not all encompassing and other methods are reported in the literature and can be used, *e.g.*:

(1) CAVEAT: Bartlett *et* al.,(1989), in Chemical and Biological Problems in Molecular Recognition, Roberts, S.M.; Ley, S.V.; Campbell, M.M. eds.; *Royal*

Society of Chemistry: Cambridge, pp.182-196.

(2) FLOG: Miller et al., (1994), *J. Comp. Aided Molec. Design* 8:153.

(3) PRO Modulator: Clark *et al.*, (1995), *J. Comp. Aided Molec. Design* 9:13.

(4) MCSS: Miranker and Karplus, (1991), *Proteins: Structure, Function, and Genetics 11:29.*

(5) AUTODOCK: Goodsell and Olson, (1990), *Proteins: Structure, Function, and Genetics 8:195.*

(6) GRID: Goodford, (1985), *J. Med. Chem.* 28:849.

Design by Modifying Compounds in Complex with PDE5A

[0168] Another way of identifying compounds as potential modulators is to modify an existing modulator in the polypeptide active site. For example, the computer representation of modulators can be modified within the computer representation of a PDE5A active site. Detailed instructions for this technique can be found, for example, in the Accelerys User Manual, 1995 in LUDI. The computer representation of the modulator is typically modified by the deletion of a chemical group or groups or by the addition of a chemical group or groups.

[0169] Upon each modification to the compound, the atoms of the modified compound and active site can be shifted in conformation and the distance between the modulator and the active-site atoms may be scored along with any complementary interactions formed between the two molecules. Scoring can be complete when a favorable geometric fit and favorable complementary interactions are attained. Compounds that have favorable scores are potential modulators.

Design by Modifying the Structure of Compounds that Bind PDE5A

[0170] A third method of structure-based modulator design is to screen compounds designed by a modulator building or modulator searching computer program. Examples of these types of programs can be found in the Molecular Simulations Package, Catalyst. Descriptions for using this program are documented in the Molecular Simulations User Guide (1995). Other computer programs used in this application are ISIS/HOST, ISIS/BASE, ISIS/DRAW) from Molecular Designs Limited and UNITY from Tripos Associates.

[0171] These programs can be operated on the structure of a compound that has been removed from the active site of the three dimensional structure of a compound-phosphodiesterase complex. Operating the program on such a compound is preferable since it is in a biologically active conformation.

[0172] A modulator construction computer program is a computer program that may be used to replace computer representations of chemical groups in a compound complexed with a phosphodiesterase or other biomolecule with groups from a computer database. A modulator searching computer program is a computer program that may be used to search computer representations of compounds from a computer data base that have similar three dimensional structures and similar chemical groups as compound bound to a particular biomolecule.

[0173] A typical program can operate by using the following general steps:

(a) map the compounds by chemical features such as by hydrogen bond donors or acceptors, hydrophobic/lipophilic sites, positively ionizable sites, or negatively ionizable sites;

(b) add geometric constraints to the mapped features; and

(c) search databases with the model generated in (b).

[0174] Those skilled in the art also recognize that not all of the possible chemical features of the compound need be present in the model of (b). One can use any subset of the model to generate different models for data base searches.

Modulator Design Using Molecular Scaffolds

[0175] The present invention can also advantageously utilize methods for designing compounds, designated as molecular scaffolds, that can act broadly across families of molecules and/or for using a molecular scaffold to design ligands that target individual or multiple members of those families. Such design using molecular scaffolds is described in Hirth and Milburn, U.S. Patent Application 10/377,268, which is incorporated herein by reference in its entirety. Such design and development using molecular scaffolds is described, in part, below.

[0176] In preferred embodiments, the molecules can be proteins and a set of chemical compounds can be assembled that have properties such that they are 1) chemically

designed to act on certain protein families and/or 2) behave more like molecular scaffolds, meaning that they have chemical substructures that make them specific for binding to one or more proteins in a family of interest. Alternatively, molecular scaffolds can be designed that are preferentially active on an individual target molecule.

[0177] Useful chemical properties of molecular scaffolds can include one or more of the following characteristics, but are not limited thereto: an average molecular weight below about 350 daltons, or between from about 150 to about 350 daltons, or from about 150 to about 300 daltons; having a clogP below 3; a number of rotatable bonds of less than 4; a number of hydrogen bond donors and acceptors below 5 or below 4; a polar surface area of less than 50 $Å^2$; binding at protein binding sites in an orientation so that chemical substituents from a combinatorial library that are attached to the scaffold can be projected into pockets in the protein binding site; and possessing chemically tractable structures at its substituent attachment points that can be modified, thereby enabling rapid library construction.

[0178] By "clog P" is meant the calculated log P of a compound, "P" referring to the partition coefficient between octanol and water.

[0179] The term "Molecular Polar Surface Area (PSA)" refers to the sum of surface contributions of polar atoms (usually oxygens, nitrogens and attached hydrogens) in a molecule. The polar surface area has been shown to correlate well with drug transport properties, such as intestinal absorption, or blood-brain barrier penetration.

[0180] Additional useful chemical properties of distinct compounds for inclusion in a combinatorial library include the ability to attach chemical moieties to the compound that will not interfere with binding of the compound to at least one protein of interest, and that will impart desirable properties to the library members, for example, causing the library members to be actively transported to cells and/or organs of interest, or the ability to attach to a device such as a chromatography column (*e.g.*, a streptavidin column through a molecule such as biotin) for uses such as tissue and proteomics profiling purposes.

[0181] A person of ordinary skill in the art will realize other properties that can be desirable for the scaffold or library members to have depending on the particular requirements of the use, and that compounds with these properties can also be sought and

identified in like manner. Methods of selecting compounds for assay are known to those of ordinary skill in the art, for example, methods and compounds described in U.S. Patent No. 6,288,234, 6,090,912, 5,840,485, each of which is hereby incorporated by reference in its entirety, including all charts and drawings.

[0182] In various embodiments, the present invention provides methods of designing ligands that bind to a plurality of members of a molecular family, where the ligands contain a common molecular scaffold. Thus, a compound set can be assayed for binding to a plurality of members of a molecular family, *e.g.*, a protein family. One or more compounds that bind to a plurality of family members can be identified as molecular scaffolds. When the orientation of the scaffold at the binding site of the target molecules has been determined and chemically tractable structures have been identified, a set of ligands can be synthesized starting with one or a few molecular scaffolds to arrive at a plurality of ligands, wherein each ligand binds to a separate target molecule of the molecular family with altered or changed binding affinity or binding specificity relative to the scaffold. Thus, a plurality of drug lead molecules can be designed to preferentially target individual members of a molecular family based on the same molecular scaffold, and act on them in a specific manner.

IX. Binding Assays

[0183] The methods of the present invention can involve assays that are able to detect the binding of compounds to a target molecule. Such binding is at a statistically significant level, preferably with a confidence level of at least 90%, more preferably at least 95, 97, 98, 99% or greater confidence level that the assay signal represents binding to the target molecule, *i.e.*, is distinguished from background. Preferably controls are used to distinguish target binding from non-specific binding. The assays of the present invention can also include assaying compounds for low affinity binding to the target molecule. A large variety of assays indicative of binding are known for different target types and can be used for this invention. Compounds that act broadly across protein families are not likely to have a high affinity against individual targets, due to the broad nature of their binding. Thus, assays described herein allow for the identification of compounds that bind with low affinity, very low affinity, and extremely low affinity. Therefore, potency (or binding affinity) is not the primary, nor even the most important, indicia of identification of a potentially useful binding compound. Rather, even those compounds that bind with

low affinity, very low affinity, or extremely low affinity can be considered as molecular scaffolds that can continue to the next phase of the ligand design process.

[0184] By binding with "low affinity" is meant binding to the target molecule with a dissociation constant (k_d) of greater than 1 μM under standard conditions. By binding with "very low affinity" is meant binding with a k_d of above about 100 μM under standard conditions. By binding with "extremely low affinity" is meant binding at a k_d of above about 1 mM under standard conditions. By "moderate affinity" is meant binding with a k_d of from about 200 nM to about 1 μM under standard conditions. By "moderately high affinity" is meant binding at a k_d of from about 1 nM to about 200 nM. By binding at "high affinity" is meant binding at a k_d of below about 1 nM under standard conditions. For example, low affinity binding can occur because of a poorer fit into the binding site of the target molecule or because of a smaller number of non-covalent bonds, or weaker covalent bonds present to cause binding of the scaffold or ligand to the binding site of the target molecule relative to instances where higher affinity binding occurs. The standard conditions for binding are at pH 7.2 at 37°C for one hour. For example, 100 μl/well can be used in HEPES 50 mM buffer at pH 7.2, NaCl 15 mM, ATP 2 μM, and bovine serum albumin 1 ug/well, 37°C for one hour.

[0185] Binding compounds can also be characterized by their effect on the activity of the target molecule. Thus, a "low activity" compound has an inhibitory concentration (IC_{50}) or excitation concentration (EC_{50}) of greater than 1 μM under standard conditions. By "very low activity" is meant an IC_{50} or EC_{50} of above 100 μM under standard conditions. By "extremely low activity" is meant an IC_{50} or EC_{50} of above 1 mM under standard conditions. By "moderate activity" is meant an IC_{50} or EC_{50} of 200 nM to 1 μM under standard conditions. By "moderately high activity" is meant an IC_{50} or EC_{50} of 1 nM to 200 nM. By "high activity" is meant an IC_{50} or EC_{50} of below 1 nM under standard conditions. The IC_{50} (or EC_{50}) is defined as the concentration of compound at which 50% of the activity of the target molecule (e.g., enzyme or other protein) activity being measured is lost (or gained) relative to activity when no compound is present. Activity can be measured using methods known to those of ordinary skill in the art, *e.g.*, by measuring any detectable product or signal produced by occurrence of an enzymatic reaction, or other activity by a protein being measured.

[0186] By "background signal" in reference to a binding assay is meant the signal that is recorded under standard conditions for the particular assay in the absence of a test compound, molecular scaffold, or ligand that binds to the target molecule. Persons of ordinary skill in the art will realize that accepted methods exist and are widely available for determining background signal.

[0187] By "standard deviation" is meant the square root of the variance. The variance is a measure of how spread out a distribution is. It is computed as the average squared deviation of each number from its mean. For example, for the numbers 1, 2, and 3, the mean is 2 and the variance is:

$$\sigma^2 = \frac{(1-2)^2 + (2-2)^2 + (3-2)^2}{3} = 0.667$$

[0188] To design or discover scaffolds that act broadly across protein families, proteins of interest can be assayed against a compound collection or set. The assays can preferably be enzymatic or binding assays. In some embodiments it may be desirable to enhance the solubility of the compounds being screened and then analyze all compounds that show activity in the assay, including those that bind with low affinity or produce a signal with greater than about three times the standard deviation of the background signal. The assays can be any suitable assay such as, for example, binding assays that measure the binding affinity between two binding partners. Various types of screening assays that can be useful in the practice of the present invention are known in the art, such as those described in U.S. Patent Nos. 5,763,198, 5,747,276, 5,877,007, 6,243,980, 6,294,330, and 6,294,330, each of which is hereby incorporated by reference in its entirety, including all charts and drawings.

[0189] In various embodiments of the assays at least one compound, at least about 5%, at least about 10%, at least about 15%, at least about 20%, or at least about 25% of the compounds can bind with low affinity. In general, up to about 20% of the compounds can show activity in the screening assay and these compounds can then be analyzed directly with high-throughput co-crystallography, computational analysis to group the compounds into classes with common structural properties (e.g., structural core and/or shape and polarity characteristics), and the identification of common chemical structures between compounds that show activity.

[0190] The person of ordinary skill in the art will realize that decisions can be based on criteria that are appropriate for the needs of the particular situation, and that the decisions can be made by computer software programs. Classes can be created containing almost any number of scaffolds, and the criteria selected can be based on increasingly exacting criteria until an arbitrary number of scaffolds is arrived at for each class that is deemed to be advantageous.

Surface Plasmon Resonance

[0191] Binding parameters can be measured using surface plasmon resonance, for example, with a BIAcore® chip (Biacore, Japan) coated with immobilized binding components. Surface plasmon resonance is used to characterize the microscopic association and dissociation constants of reaction between an sFv or other ligand directed against target molecules. Such methods are generally described in the following references which are incorporated herein by reference. Vely F. et al., (2000) BIAcore® analysis to test phosphopeptide-SH2 domain interactions, *Methods in Molecular Biology.* 121:313-21; Liparoto et al., (1999) Biosensor analysis of the interleukin-2 receptor complex, *Journal of Molecular Recognition.* 12:316-21; Lipschultz et al., (2000) Experimental design for analysis of complex kinetics using surface plasmon resonance, *Methods.* 20(3):310-8; Malmqvist., (1999) BIACORE: an affinity biosensor system for characterization of biomolecular interactions, Biochemical Society Transactions 27:335-40; Alfthan, (1998) Surface plasmon resonance biosensors as a tool in antibody engineering, *Biosensors & Bioelectronics.* 13:653-63; Fivash et al., (1998) BIAcore for macromolecular interaction, Current Opinion in Biotechnology. 9:97-101; Price et al.; (1998) Summary report on the ISOBM TD-4 Workshop: analysis of 56 monoclonal antibodies against the MUC1 mucin. *Tumour Biology* 19 Suppl 1:1-20; Malmqvist et al, (1997) Biomolecular interaction analysis: affinity biosensor technologies for functional analysis of proteins, *Current Opinion in Chemical Biology.* 1:378-83; O'Shannessy et al., (1996) Interpretation of deviations from pseudo-first-order kinetic behavior in the characterization of ligand binding by biosensor technology, *Analytical Biochemistry.* 236:275-83; Malmborg et al., (1995) BIAcore as a tool in antibody engineering, *Journal of Immunological Methods.* 183:7-13; Van Regenmortel, (1994) Use of biosensors to characterize recombinant proteins, *Developments in Biological Standardization.* 83:143-51; and O'Shannessy, (1994) Determination of kinetic rate and equilibrium binding

constants for macromolecular interactions: a critique of the surface plasmon resonance literature, *Current Opinions in Biotechnology*. 5:65-71.

[0192] BIAcore® uses the optical properties of surface plasmon resonance (SPR) to detect alterations in protein concentration bound to a dextran matrix lying on the surface of a gold/glass sensor chip interface, a dextran biosensor matrix. In brief, proteins are covalently bound to the dextran matrix at a known concentration and a ligand for the protein is injected through the dextran matrix. Near infrared light, directed onto the opposite side of the sensor chip surface is reflected and also induces an evanescent wave in the gold film, which in turn, causes an intensity dip in the reflected light at a particular angle known as the resonance angle. If the refractive index of the sensor chip surface is altered (e.g., by ligand binding to the bound protein) a shift occurs in the resonance angle. This angle shift can be measured and is expressed as resonance units (RUs) such that 1000 RUs is equivalent to a change in surface protein concentration of 1 ng/mm^2. These changes are displayed with respect to time along the y-axis of a sensorgram, which depicts the association and dissociation of any biological reaction.

High Throughput Screening (HTS) Assays

[0193] HTS typically uses automated assays to search through large numbers of compounds for a desired activity. Typically HTS assays are used to find new drugs by screening for chemicals that act on a particular enzyme or molecule. For example, if a chemical inactivates an enzyme it might prove to be effective in preventing a process in a cell which causes a disease. High throughput methods enable researchers to assay thousands of different chemicals against each target molecule very quickly using robotic handling systems and automated analysis of results.

[0194] As used herein, "high throughput screening" or "HTS" refers to the rapid in vitro screening of large numbers of compounds (libraries); generally tens to hundreds of thousands of compounds, using robotic screening assays. Ultra high-throughput Screening (uHTS) generally refers to the high-throughput screening accelerated to greater than 100,000 tests per day.

[0195] To achieve high-throughput screening, it is advantageous to house samples on a multicontainer carrier or platform. A multicontainer carrier facilitates measuring reactions of a plurality of candidate compounds simultaneously. Multi-well microplates may be

used as the carrier. Such multi-well microplates, and methods for their use in numerous assays, are both known in the art and commercially available.

[0196] Screening assays may include controls for purposes of calibration and confirmation of proper manipulation of the components of the assay. Blank wells that contain all of the reactants but no member of the chemical library are usually included. As another example, a known inhibitor (or activator) of an enzyme for which modulators are sought, can be incubated with one sample of the assay, and the resulting decrease (or increase) in the enzyme activity used as a comparator or control. It will be appreciated that modulators can also be combined with the enzyme activators or inhibitors to find modulators which inhibit the enzyme activation or repression that is otherwise caused by the presence of the known the enzyme modulator. Similarly, when ligands to a sphingolipid target are sought, known ligands of the target can be present in control/calibration assay wells.

Measuring Enzymatic and Binding Reactions During Screening Assays

[0197] Techniques for measuring the progression of enzymatic and binding reactions, e.g., in multicontainer carriers, are known in the art and include, but are not limited to, the following.

[0198] Spectrophotometric and spectrofluorometric assays are well known in the art. Examples of such assays include the use of colorimetric assays for the detection of peroxides, as disclosed in Example 1(b) and Gordon, A. J. and Ford, R. A., (1972) The Chemist's Companion: A Handbook Of Practical Data, Techniques, And References, John Wiley and Sons, N.Y., Page 437.

[0199] Fluorescence spectrometry may be used to monitor the generation of reaction products. Fluorescence methodology is generally more sensitive than the absorption methodology. The use of fluorescent probes is well known to those skilled in the art. For reviews, see Bashford et al., (1987) Spectrophotometry and Spectrofluorometry: A Practical Approach, pp. 91-114, IRL Press Ltd.; and Bell, (1981) Spectroscopy In Biochemistry, Vol. I, pp. 155-194, CRC Press.

[0200] In spectrofluorometric methods, enzymes are exposed to substrates that change their intrinsic fluorescence when processed by the target enzyme. Typically, the substrate

is nonfluorescent and is converted to a fluorophore through one or more reactions. As a non-limiting example, SMase activity can be detected using the Amplex® Red reagent (Molecular Probes, Eugene, OR). In order to measure sphingomyelinase activity using Amplex® Red, the following reactions occur. First, SMase hydrolyzes sphingomyelin to yield ceramide and phosphorylcholine. Second, alkaline phosphatase hydrolyzes phosphorylcholine to yield choline. Third, choline is oxidized by choline oxidase to betaine. Finally, H_2O_2, in the presence of horseradish peroxidase, reacts with Amplex® Red to produce the fluorescent product, Resorufin, and the signal therefrom is detected using spectrofluorometry.

[0201] Fluorescence polarization (FP) is based on a decrease in the speed of molecular rotation of a fluorophore that occurs upon binding to a larger molecule, such as a receptor protein, allowing for polarized fluorescent emission by the bound ligand. FP is empirically determined by measuring the vertical and horizontal components of fluorophore emission following excitation with plane polarized light. Polarized emission is increased when the molecular rotation of a fluorophore is reduced. A fluorophore produces a larger polarized signal when it is bound to a larger molecule (i.e. a receptor), slowing molecular rotation of the fluorophore. The magnitude of the polarized signal relates quantitatively to the extent of fluorescent ligand binding. Accordingly, polarization of the "bound" signal depends on maintenance of high affinity binding.

[0202] FP is a homogeneous technology and reactions are very rapid, taking seconds to minutes to reach equilibrium. The reagents are stable, and large batches may be prepared, resulting in high reproducibility. Because of these properties, FP has proven to be highly automatable, often performed with a single incubation with a single, premixed, tracer-receptor reagent. For a review, see Owickiet al., (1997), Application of Fluorescence Polarization Assays in High-Throughput Screening, *Genetic Engineering News*, 17:27.

[0203] FP is particularly desirable since its readout is independent of the emission intensity (Checovich, W. J., et al., (1995) *Nature* 375:254-256; Dandliker, W. B., et al., (1981) *Methods in Enzymology* 74:3-28) and is thus insensitive to the presence of colored compounds that quench fluorescence emission. FP and FRET (see below) are well-suited for identifying compounds that block interactions between sphingolipid receptors and their ligands. See, for example, Parker et al., (2000) Development of high throughput screening

assays using fluorescence polarization: nuclear receptor-ligand-binding and kinase/phosphatase assays, J Biomol Screen 5:77-88.

[0204] Fluorophores derived from sphingolipids that may be used in FP assays are commercially available. For example, Molecular Probes (Eugene, OR) currently sells sphingomyelin and one ceramide flurophores. These are, respectively, N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene- 3-pentanoyl)sphingosyl phosphocholine (BODIPY® FL C5-sphingomyelin); N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene- 3-dodecanoyl)sphingosyl phosphocholine (BODIPY® FL C12-sphingomyelin); and N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene- 3-pentanoyl)sphingosine (BODIPY® FL C5-ceramide). U.S. Patent No. 4,150,949, (Immunoassay for gentamicin), discloses fluorescein-labelled gentamicins, including fluoresceinthiocarbanyl gentamicin. Additional fluorophores may be prepared using methods well known to the skilled artisan.

[0205] Exemplary normal-and-polarized fluorescence readers include the POLARION® fluorescence polarization system (Tecan AG, Hombrechtikon, Switzerland). General multiwell plate readers for other assays are available, such as the VERSAMAX® reader and the SPECTRAMAX® multiwell plate spectrophotometer (both from Molecular Devices).

[0206] Fluorescence resonance energy transfer (FRET) is another useful assay for detecting interaction and has been described. See, e.g., Heim et al., (1996) *Curr. Biol.* 6:178-182; Mitra et al., (1996) *Gene* 173:13-17; and Selvin et al., (1995) *Meth. Enzymol.* 246:300-345. FRET detects the transfer of energy between two fluorescent substances in close proximity, having known excitation and emission wavelengths. As an example, a protein can be expressed as a fusion protein with green fluorescent protein (GFP). When two fluorescent proteins are in proximity, such as when a protein specifically interacts with a target molecule, the resonance energy can be transferred from one excited molecule to the other. As a result, the emission spectrum of the sample shifts, which can be measured by a fluorometer, such as a fMAX multiwell fluorometer (Molecular Devices, Sunnyvale Calif.).

[0207] Scintillation proximity assay (SPA) is a particularly useful assay for detecting an interaction with the target molecule. SPA is widely used in the pharmaceutical industry and has been described (Hanselman et al., (1997) *J. Lipid Res.* 38:2365-2373; Kahl et al.,

(1996) *Anal. Biochem.* 243:282-283; Undenfriend et al., (1987) *Anal. Biochem.* 161:494-500). See also U.S. Patent Nos. 4,626,513 and 4,568,649, and European Patent No. 0,154,734. One commercially available system uses FLASHPLATE® scintillant-coated plates (NEN Life Science Products, Boston, MA).

[0208] The target molecule can be bound to the scintillator plates by a variety of well known means. Scintillant plates are available that are derivatized to bind to fusion proteins such as GST, His6 or Flag fusion proteins. Where the target molecule is a protein complex or a multimer, one protein or subunit can be attached to the plate first, then the other components of the complex added later under binding conditions, resulting in a bound complex.

[0209] In a typical SPA assay, the gene products in the expression pool will have been radiolabeled and added to the wells, and allowed to interact with the solid phase, which is the immobilized target molecule and scintillant coating in the wells. The assay can be measured immediately or allowed to reach equilibrium. Either way, when a radiolabel becomes sufficiently close to the scintillant coating, it produces a signal detectable by a device such as a TOPCOUNT NXT® microplate scintillation counter (Packard BioScience Co., Meriden Conn.). If a radiolabeled expression product binds to the target molecule, the radiolabel remains in proximity to the scintillant long enough to produce a detectable signal.

[0210] In contrast, the labeled proteins that do not bind to the target molecule, or bind only briefly, will not remain near the scintillant long enough to produce a signal above background. Any time spent near the scintillant caused by random Brownian motion will also not result in a significant amount of signal. Likewise, residual unincorporated radiolabel used during the expression step may be present, but will not generate significant signal because it will be in solution rather than interacting with the target molecule. These non-binding interactions will therefore cause a certain level of background signal that can be mathematically removed. If too many signals are obtained, salt or other modifiers can be added directly to the assay plates until the desired specificity is obtained (Nichols et al., (1998) *Anal. Biochem.* 257:112-119).

Assay Compounds and Molecular Scaffolds

[0211] Preferred characteristics of a scaffold include being of low molecular weight (e.g., less than 350 Da, or from about 100 to about 350 daltons, or from about 150 to about 300 daltons). Preferably clog P of a scaffold is from –1 to 8, more preferably less than 6, 5, or 4, most preferably less than 3. In particular embodiments the clogP is in a range –1 to an upper limit of 2, 3, 4, 5, 6, or 8; or is in a range of 0 to an upper limit of 2,3, 4, 5, 6, or 8. Preferably the number of rotatable bonds is less than 5, more preferably less than 4. Preferably the number of hydrogen bond donors and acceptors is below 6, more preferably below 5. An additional criterion that can be useful is a polar surface area of less than 5. Guidance that can be useful in identifying criteria for a particular application can be found in Lipinski et al., (1997) *Advanced Drug Delivery Reviews* 23 3-25, which is hereby incorporated by reference in its entirety.

[0212] A scaffold may preferably bind to a given protein binding site in a configuration that causes substituent moieties of the scaffold to be situated in pockets of the protein binding site. Also, possessing chemically tractable groups that can be chemically modified, particularly through synthetic reactions, to easily create a combinatorial library can be a preferred characteristic of the scaffold. Also preferred can be having positions on the scaffold to which other moieties can be attached, which do not interfere with binding of the scaffold to the protein(s) of interest but do cause the scaffold to achieve a desirable property, for example, active transport of the scaffold to cells and/or organs, enabling the scaffold to be attached to a chromatographic column to facilitate analysis, or another desirable property. A molecular scaffold can bind to a target molecule with any affinity, such as binding at high affinity, moderate affinity, low affinity, very low affinity, or extremely low affinity.

[0213] Thus, the above criteria can be utilized to select many compounds for testing that have the desired attributes. Many compounds having the criteria described are available in the commercial market, and may be selected for assaying depending on the specific needs to which the methods are to be applied.

[0214] A "compound library" or "library" is a collection of different compounds having different chemical structures. A compound library is screenable, that is, the compound library members therein may be subject to screening assays. In preferred embodiments,

the library members can have a molecular weight of from about 100 to about 350 daltons, or from about 150 to about 350 daltons. Examples of libraries are provided aove.

[0215] Libraries of the present invention can contain at least one compound than binds to the target molecule at low affinity. Libraries of candidate compounds can be assayed by many different assays, such as those described above, e.g., a fluorescence polarization assay. Libraries may consist of chemically synthesized peptides, peptidomimetics, or arrays of combinatorial chemicals that are large or small, focused or nonfocused. By "focused" it is meant that the collection of compounds is prepared using the structure of previously characterized compounds and/or pharmacophores.

[0216] Compound libraries may contain molecules isolated from natural sources, artificially synthesized molecules, or molecules synthesized, isolated, or otherwise prepared in such a manner so as to have one or more moieties variable, e.g., moieties that are independently isolated or randomly synthesized. Types of molecules in compound libraries include but are not limited to organic compounds, polypeptides and nucleic acids as those terms are used herein, and derivatives, conjugates and mixtures thereof.

[0217] Compound libraries of the invention may be purchased on the commercial market or prepared or obtained by any means including, but not limited to, combinatorial chemistry techniques, fermentation methods, plant and cellular extraction procedures and the like (see, e.g., Cwirla et al., (1990) *Biochemistry*, 87, 6378-6382; Houghten et al., (1991) *Nature*, 354, 84-86; Lam et al., (1991) *Nature*, 354, 82-84; Brenner et al., (1992) *Proc. Natl. Acad. Sci. USA*, 89, 5381-5383; R. A. Houghten, (1993) *Trends Genet.*, 9, 235-239; E. R. Felder, (1994) *Chimia*, 48, 512-541; Gallop et al., (1994) *J. Med. Chem.*, 37, 1233-1251; Gordon et al., (1994) *J. Med. Chem.*, 37, 1385-1401; Carell et al., (1995) *Chem. Biol.*, 3, 171-183; Madden et al., *Perspectives in Drug Discovery and Design* 2, 269-282; Lebl et al., (1995) *Biopolymers*, 37 177-198); small molecules assembled around a shared molecular structure; collections of chemicals that have been assembled by various commercial and noncommercial groups, natural products; extracts of marine organisms, fungi, bacteria, and plants.

[0218] Preferred libraries can be prepared in a homogenous reaction mixture, and separation of unreacted reagents from members of the library is not required prior to screening. Although many combinatorial chemistry approaches are based on solid state

chemistry, liquid phase combinatorial chemistry is capable of generating libraries (Sun CM., (1999) Recent advances in liquid-phase combinatorial chemistry, *Combinatorial Chemistry & High Throughput Screening.* 2:299-318).

[0219] Libraries of a variety of types of molecules are prepared in order to obtain members therefrom having one or more preselected attributes that can be prepared by a variety of techniques, including but not limited to parallel array synthesis (Houghton, (2000) *Annu Rev Pharmacol Toxicol* 40:273-82, Parallel array and mixture-based synthetic combinatorial chemistry; solution-phase combinatorial chemistry (Merritt, (1998) *Comb Chem High Throughput Screen* 1(2):57-72, Solution phase combinatorial chemistry, Coe et al., (1998-99) *Mol Divers*;4(1):31-8, Solution-phase combinatorial chemistry, Sun, (1999) *Comb Chem High Throughput Screen* 2(6):299-318, Recent advances in liquid-phase combinatorial chemistry); synthesis on soluble polymer (Gravert et al., (1997) *Curr Opin Chem Biol* 1(1):107-13, Synthesis on soluble polymers: new reactions and the construction of small molecules); and the like. See, e.g., Dolle et al., (1999) *J Comb Chem* 1(4):235-82, Comprehensive survey of cominatorial library synthesis: 1998. Freidinger RM., (1999) Nonpeptidic ligands for peptide and protein receptors, Current Opinion in Chemical Biology; and Kundu et al., *Prog Drug Res*;53:89-156, Combinatorial chemistry: polymer supported synthesis of peptide and non-peptide libraries). Compounds may be clinically tagged for ease of identification (Chabala, (1995) *Curr Opin Biotechnol* 6(6):633-9, Solid-phase combinatorial chemistry and novel tagging methods for identifying leads).

[0220] The combinatorial synthesis of carbohydrates and libraries containing oligosaccharides have been described (Schweizer et al., (1999) *Curr* Opin *Chem Biol* 3(3):291-8, Combinatorial synthesis of carbohydrates). The synthesis of natural-product based compound libraries has been described (Wessjohann, (2000) *Curr Opin Chem Biol* 4(3):303-9, Synthesis of natural-product based compound libraries).

[0221] Libraries of nucleic acids are prepared by various techniques, including by way of non-limiting example the ones described herein, for the isolation of aptamers. Libraries that include oligonucleotides and polyaminooligonucleotides (Markiewicz et al., (2000) Synthetic oligonucleotide combinatorial libraries and their applications, *Farmaco.* 55:174-7) displayed on streptavidin magnetic beads are known. Nucleic acid libraries are known

that can be coupled to parallel sampling and be deconvoluted without complex procedures such as automated mass spectrometry (Enjalbal C. Martinez J. Aubagnac JL, (2000) Mass spectrometry in combinatorial chemistry, *Mass Spectrometry Reviews*. 19:139-61) and parallel tagging. (Perrin DM., Nucleic acids for recognition and catalysis: landmarks, limitations, and looking to the future, *Combinatorial Chemistry & High Throughput Screening* 3:243-69).

[0222] Peptidomimetics are identified using combinatorial chemistry and solid phase synthesis (Kim HO. Kahn M., (2000) A merger of rational drug design and combinatorial chemistry: development and application of peptide secondary structure mimetics, Combinatorial Chemistry & High Throughput Screening 3:167-83; al-Obeidi, (1998) *Mol Biotechnol* 9(3):205-23, Peptide and peptidomimetric libraries. Molecular diversity and drug design). The synthesis may be entirely random or based in part on a known polypeptide.

[0223] Polypeptide libraries can be prepared according to various techniques. In brief, phage display techniques can be used to produce polypeptide ligands (Gram H., (1999) Phage display in proteolysis and signal transduction, Combinatorial Chemistry & High Throughput Screening. 2:19-28) that may be used as the basis for synthesis of peptidomimetics. Polypeptides, constrained peptides, proteins, protein domains, antibodies, single chain antibody fragments, antibody fragments, and antibody combining regions are displayed on filamentous phage for selection.

[0224] Large libraries of individual variants of human single chain Fv antibodies have been produced. See, e.g., Siegel RW. Allen B. Pavlik P. Marks JD. Bradbury A., (2000) Mass spectral analysis of a protein complex using single-chain antibodies selected on a peptide target: applications to functional genomics, *Journal of Molecular Biology* 302:285-93; Poul MA. Becerril B. Nielsen UB. Morisson P. Marks JD.,(2000) Selection of tumor-specific internalizing human antibodies from phage libraries. Source *Journal of Molecular Biology*. 301:1149-61; Amersdorfer P. Marks JD., (2001) Phage libraries for generation of anti-botulinum scFv antibodies, *Methods in Molecular Biology*. 145:219-40; Hughes-Jones NC. Bye JM. Gorick BD. Marks JD. Ouwehand WH., (1999) Synthesis of Rh Fv phage-antibodies using VH and VL germline genes, *British Journal of Haematology*. 105:811-6; McCall AM. Amoroso AR. Sautes C. Marks JD. Weiner LM.,

(1998) Characterization of anti-mouse Fc gamma RII single-chain Fv fragments derived from human phage display libraries, *Immunotechnology*. 4:71-87; Sheets MD. Amersdorfer P. Finnern R. Sargent P. Lindquist E. Schier R. Hemingsen G. Wong C. Gerhart JC. Marks JD. Lindquist E., (1998) Efficient construction of a large nonimmune phage antibody library: the production of high-affinity human single-chain antibodies to protein antigens (published erratum appears in *Proc Natl Acad Sci* USA 1999 96:795), *Proc Natl Acad Sci USA* 95:6157-62).

[0225] Focused or smart chemical and pharmacophore libraries can be designed with the help of sophisticated strategies involving computational chemistry (e.g., Kundu B. Khare SK. Rastogi SK., (1999) Combinatorial chemistry: polymer supported synthesis of peptide and non-peptide libraries, *Progress in Drug Research* 53:89-156) and the use of structure-based ligands using database searching and docking, de novo drug design and estimation of ligand binding affinities (Joseph-McCarthy D., (1999) Computational approaches to structure-based ligand design, *Pharmacology & Therapeutics* 84:179-91; Kirkpatrick DL. Watson S. Ulhaq S., (1999) Structure-based drug design: combinatorial chemistry and molecular modeling, *Combinatorial Chemistry & High Throughput Screening*. 2:211-21; Eliseev AV. Lehn JM., (1999) Dynamic combinatorial chemistry: evolutionary formation and screening of molecular libraries, *Current Topics in Microbiology & Immunology* 243:159-72; Bolger et al., (1991) *Methods Enz.* 203:21-45; Martin, (1991) *Methods Enz.* 203:587-613; Neidle et al., (1991) *Methods Enz.* 203:433-458; U.S. Patent 6,178,384).

X. Crystallography

[0226] After binding compounds have been determined, the orientation of compound bound to target is determined. Preferably this determination involves crystallography on co-crystals of molecular scaffold compounds with target. Most protein crystallographic platforms can preferably be designed to analyze up to about 500 co-complexes of compounds, ligands, or molecular scaffolds bound to protein targets due to the physical parameters of the instruments and convenience of operation. If the number of scaffolds that have binding activity exceeds a number convenient for the application of crystallography methods, the scaffolds can be placed into groups based on having at least one common chemical structure or other desirable characteristics, and representative compounds can be selected from one or more of the classes. Classes can be made with

increasingly exacting criteria until a desired number of classes (e.g., 500) is obtained. The classes can be based on chemical structure similarities between molecular scaffolds in the class, e.g., all possess a pyrrole ring, benzene ring, or other chemical feature. Likewise, classes can be based on shape characteristics, e.g., space-filling characteristics.

[0227] The co-crystallography analysis can be performed by co-complexing each scaffold with its target at concentrations of the scaffold that showed activity in the screening assay. This co-complexing can be accomplished with the use of low percentage organic solvents with the target molecule and then concentrating the target with each of the scaffolds. In preferred embodiments these solvents are less than 5% organic solvent such as dimethyl sulfoxide (DMSO), ethanol, methanol, or ethylene glycol in water or another aqueous solvent. Each scaffold complexed to the target molecule can then be screened with a suitable number of crystallization screening conditions at both 4 and 20 degrees. In preferred embodiments, about 96 crystallization screening conditions can be performed in order to obtain sufficient information about the co-complexation and crystallization conditions, and the orientation of the scaffold at the binding site of the target molecule. Crystal structures can then be analyzed to determine how the bound scaffold is oriented physically within the binding site or within one or more binding pockets of the molecular family member.

[0228] It is desirable to determine the atomic coordinates of the compounds bound to the target proteins in order to determine which is a most suitable scaffold for the protein family. X-ray crystallographic analysis is therefore most preferable for determining the atomic coordinates. Those compounds selected can be further tested with the application of medicinal chemistry. Compounds can be selected for medicinal chemistry testing based on their binding position in the target molecule. For example, when the compound binds at a binding site, the compound's binding position in the binding site of the target molecule can be considered with respect to the chemistry that can be performed on chemically tractable structures or sub-structures of the compound, and how such modifications on the compound might interact with structures or sub-structures on the binding site of the target. Thus, one can explore the binding site of the target and the chemistry of the scaffold in order to make decisions on how to modify the scaffold to arrive at a ligand with higher potency and/or selectivity. This process allows for more direct design of ligands, by utilizing structural and chemical information obtained directly

from the co-complex, thereby enabling one to more efficiently and quickly design lead compounds that are likely to lead to beneficial drug products. In various embodiments it may be desirable to perform co-crystallography on all scaffolds that bind, or only those that bind with a particular affinity, for example, only those that bind with high affinity, moderate affinity, low affinity, very low affinity, or extremely low affinity. It may also be advantageous to perform co-crystallography on a selection of scaffolds that bind with any combination of affinities.

[0229] Standard X-ray protein diffraction studies such as by using a Rigaku RU-200® (Rigaku, Tokyo, Japan) with an X-ray imaging plate detector or a synchrotron beam-line can be performed on co-crystals and the diffraction data measured on a standard X-ray detector, such as a CCD detector or an X-ray imaging plate detector.

[0230] Performing X-ray crystallography on about 200 co-crystals should generally lead to about 50 co-crystals structures, which should provide about 10 scaffolds for validation in chemistry, which should finally result in about 5 selective leads for target molecules.

Virtual Assays

[0231] Commercially available software that generates three-dimensional graphical representations of the complexed target and compound from a set of coordinates provided can be used to illustrate and study how a compound is oriented when bound to a target. (e.g., QUANTA®, Accelerys, San Diego, CA). Thus, the existence of binding pockets at the binding site of the targets can be particularly useful in the present invention. These binding pockets are revealed by the crystallographic structure determination and show the precise chemical interactions involved in binding the compound to the binding site of the target. The person of ordinary skill will realize that the illustrations can also be used to decide where chemical groups might be added, substituted, modified, or deleted from the scaffold to enhance binding or another desirable effect, by considering where unoccupied space is located in the complex and which chemical substructures might have suitable size and/or charge characteristics to fill it. The person of ordinary skill will also realize that regions within the binding site can be flexible and its properties can change as a result of scaffold binding, and that chemical groups can be specifically targeted to those regions to achieve a desired effect. Specific locations on the molecular scaffold can be considered

with reference to where a suitable chemical substructure can be attached and in which conformation, and which site has the most advantageous chemistry available.

[0232] An understanding of the forces that bind the compounds to the target proteins reveals which compounds can most advantageously be used as scaffolds, and which properties can most effectively be manipulated in the design of ligands. The person of ordinary skill will realize that steric, ionic, hydrogen bond, and other forces can be considered for their contribution to the maintenance or enhancement of the target-compound complex. Additional data can be obtained with automated computational methods, such as docking and/or Free Energy Perturbations (FEP), to account for other energetic effects such as desolvation penalties. The compounds selected can be used to generate information about the chemical interactions with the target or for elucidating chemical modifications that can enhance selectivity of binding of the compound.

[0233] Computer models, such as homology models (*i.e.*, based on a known, experimentally derived structure) can be constructed using data from the co-crystal structures. When the target molecule is a protein or enzyme, preferred co-crystal structures for making homology models contain high sequence identity in the binding site of the protein sequence being modeled, and the proteins will preferentially also be within the same class and/or fold family. Knowledge of conserved residues in active sites of a protein class can be used to select homology models that accurately represent the binding site. Homology models can also be used to map structural information from a surrogate protein where an apo or co-crystal structure exists to the target protein.

[0234] Virtual screening methods, such as docking, can also be used to predict the binding configuration and affinity of scaffolds, compounds, and/or combinatorial library members to homology models. Using this data, and carrying out "virtual experiments" using computer software can save substantial resources and allow the person of ordinary skill to make decisions about which compounds can be suitable scaffolds or ligands, without having to actually synthesize the ligand and perform co-crystallization. Decisions thus can be made about which compounds merit actual synthesis and co-crystallization. An understanding of such chemical interactions aids in the discovery and design of drugs that interact more advantageously with target proteins and/or are more selective for one

protein family member over others. Thus, applying these principles, compounds with superior properties can be discovered.

[0235] Additives that promote co-crystallization can of course be included in the target molecule formulation in order to enhance the formation of co-crystals. In the case of proteins or enzymes, the scaffold to be tested can be added to the protein formulation, which is preferably present at a concentration of approximately 1 mg/ml. The formulation can also contain between 0%-10% (v/v) organic solvent, e.g. DMSO, methanol, ethanol, propane diol, or 1,3 dimethyl propane diol (MPD) or some combination of those organic solvents. Compounds are preferably solubilized in the organic solvent at a concentration of about 10 mM and added to the protein sample at a concentration of about 100 mM. The protein-compound complex is then concentrated to a final concentration of protein of from about 5 to about 20 mg/ml. The complexation and concentration steps can conveniently be performed using a 96-well formatted concentration apparatus (e.g., Amicon Inc., Piscataway, NJ). Buffers and other reagents present in the formulation being crystallized can contain other components that promote crystallization or are compatible with crystallization conditions, such as DTT, propane diol, glycerol.

[0236] The crystallization experiment can be set-up by placing small aliquots of the concentrated protein-compound complex (1 μl) in a 96 well format and sampling under 96 crystallization conditions. (Other screening formats can also be used, *e.g.*, plates with greater than 96 wells.) Crystals can typically be obtained using standard crystallization protocols that can involve the 96 well crystallization plate being placed at different temperatures. Co-crystallization varying factors other than temperature can also be considered for each protein-compound complex if desirable. For example, atmospheric pressure, the presence or absence of light or oxygen, a change in gravity, and many other variables can all be tested. The person of ordinary skill in the art will realize other variables that can advantageously be varied and considered.

Ligand Design and Preparation

[0237] The design and preparation of ligands can be performed with or without structural and/or co-crystallization data by considering the chemical structures in common between the active scaffolds of a set. In this process structure-activity hypotheses can be formed and those chemical structures found to be present in a substantial number of the

scaffolds, including those that bind with low affinity, can be presumed to have some effect on the binding of the scaffold. This binding can be presumed to induce a desired biochemical effect when it occurs in a biological system (e.g., a treated mammal). New or modified scaffolds or combinatorial libraries derived from scaffolds can be tested to disprove the maximum number of binding and/or structure-activity hypotheses. The remaining hypotheses can then be used to design ligands that achieve a desired binding and biochemical effect.

[0238] But in many cases it will be preferred to have co-crystallography data for consideration of how to modify the scaffold to achieve the desired binding effect (e.g., binding at higher affinity or with higher selectivity). Using the case of proteins and enzymes, co-crystallography data shows the binding pocket of the protein with the molecular scaffold bound to the binding site, and it will be apparent that a modification can be made to a chemically tractable group on the scaffold. For example, a small volume of space at a protein binding site or pocket might be filled by modifying the scaffold to include a small chemical group that fills the volume. Filling the void volume can be expected to result in a greater binding affinity, or the loss of undesirable binding to another member of the protein family. Similarly, the co-crystallography data may show that deletion of a chemical group on the scaffold may decrease a hindrance to binding and result in greater binding affinity or specificity.

[0239] It can be desirable to take advantage of the presence of a charged chemical group located at the binding site or pocket of the protein. For example, a positively charged group can be complemented with a negatively charged group introduced on the molecular scaffold. This can be expected to increase binding affinity or binding specificity, thereby resulting in a more desirable ligand. In many cases, regions of protein binding sites or pockets are known to vary from one family member to another based on the amino acid differences in those regions. Chemical additions in such regions can result in the creation or elimination of certain interactions (e.g., hydrophobic, electrostatic, or entropic) that allow a compound to be more specific for one protein target over another or to bind with greater affinity, thereby enabling one to synthesize a compound with greater selectivity or affinity for a particular family member. Additionally, certain regions can contain amino acids that are known to be more flexible than others. This often occurs in amino acids contained in loops connecting elements of the secondary structure of the protein, such as

alpha helices or beta strands. Additions of chemical moieties can also be directed to these flexible regions in order to increase the likelihood of a specific interaction occurring between the protein target of interest and the compound. Virtual screening methods can also be conducted *in silico* to assess the effect of chemical additions, subtractions, modifications, and/or substitutions on compounds with respect to members of a protein family or class.

[0240] The addition, subtraction, or modification of a chemical structure or sub-structure to a scaffold can be performed with any suitable chemical moiety. For example the following moieties, which are provided by way of example and are not intended to be limiting, can be utilized: hydrogen, alkyl, alkoxy, phenoxy, alkenyl, alkynyl, phenylalkyl, hydroxyalkyl, haloalkyl, aryl, arylalkyl, alkyloxy, alkylthio, alkenylthio, phenyl, phenylalkyl, phenylalkylthio, hydroxyalkyl-thio, alkylthiocarbbamylthio, cyclohexyl, pyridyl, piperidinyl, alkylamino, amino, nitro, mercapto, cyano, hydroxyl, a halogen atom, halomethyl, an oxygen atom (e.g., forming a ketone or N-oxide) or a sulphur atom (e.g., forming a thiol, thione, di-alkylsulfoxide or sulfone) are all examples of moieties that can be utilized.

[0241] Additional examples of structures or sub-structures that may be utilized are an aryl optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, carboxamide, nitro, and ester moieties; an amine of formula $-NX_2X_3$, where X_2 and X_3 are independently selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and homocyclic or heterocyclic ring moieties; halogen or trihalomethyl; a ketone of formula $-COX_4$, where X_4 is selected from the group consisting of alkyl and homocyclic or heterocyclic ring moieties; a carboxylic acid of formula $-(X_5)_nCOOH$ or ester of formula $(X_6)_nCOOX_7$, where X_5, X_6, and X_7 and are independently selected from the group consisting of alkyl and homocyclic or heterocyclic ring moieties and where n is 0 or 1; an alcohol of formula $(X_8)_nOH$ or an alkoxy moiety of formula $-(X_8)_nOX_9$, where X_8 and X_9 are independently selected from the group consisting of saturated or unsaturated alkyl and homocyclic or heterocyclic ring moieties, wherein said ring is optionally substituted with one or more substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, nitro, and ester and where n is 0 or 1; an amide of formula $NHCOX_{10}$, where X_{10} is selected from the group consisting of alkyl,

hydroxyl, and homocyclic or heterocyclic ring moieties, wherein said ring is optionally substituted with one or more substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, nitro, and ester; SO_2, NX_{11} X_{12}, where X_{11} and X_{12} are selected from the group consisting of hydrogen, alkyl, and homocyclic or heterocyclic ring moieties; a homocyclic or heterocyclic ring moiety optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, carboxamide, nitro, and ester moieties; an aldehyde of formula -CHO; a sulfone of formula $-SO_2X_{13}$, where X_{13} is selected from the group consisting of saturated or unsaturated alkyl and homocyclic or heterocyclic ring moieties; and a nitro of formula $-NO_2$.

Identification of Attachment Sites on Molecular Scaffolds and Ligands

[0242] In addition to the identification and development of ligands for phosphodiesterases and other enzymes, determination of the orientation of a molecular scaffold or other binding compound in a binding site allows identification of energetically allowed sites for attachment of the binding molecule to another component. For such sites, any free energy change associated with the presence of the attached component should not destablize the binding of the compound to the phosphodiesterase to an extent that will disrupt the binding. Preferably, the binding energy with the attachment should be at least 4 kcal/mol., more preferably at least 6, 8, 10, 12, 15, or 20 kcal/mol. Preferably, the presence of the attachment at the particular site reduces binding energy by no more than 3, 4, 5, 8, 10, 12, or 15 kcal/mol.

[0243] In many cases, suitable attachment sites will be those that are exposed to solvent when the binding compound is bound in the binding site. In some cases, attachment sites can be used that will result in small displacements of a portion of the enzyme without an excessive energetic cost. Exposed sites can be identified in various ways. For example, exposed sites can be identified using a graphic display or 3-dimensional model. In a grahic display, such as a computer display, an image of a compound bound in a binding site can be visually inspected to reveal atoms or groups on the compound that are exposed to solvent and oriented such that attachment at such atom or group would not preclude binding of the enzyme and binding compound. Energetic costs of attachment can be calculated based on changes or distortions that would be caused by the attachment as well as entropic changes.

[0244] Many different types of components can be attached. Persons with skill are familiar with the chemistries used for various attachments. Examples of components that can be attached include, without limitation: solid phase components such as beads, plates, chips, and wells; a dlrect or indirect label; a linker, which may be a traceless linker; among others. Such linkers can themselves be attached to other components, *e.g.*, to solid phase media, labels, and/or binding moieties.

[0245] The binding energy of a compound and the effects on binding energy for attaching the molecule to another component can be calculated approximately using any of a variety of available software or by manual calculation. An example is the following:

[0246] Calculations were performed to estimate binding energies of different organic molecules to two Kinases: PIM-1 and CDK2. The organic molecules considered included Staurosporine, identified compounds that bind to PDE5A, and several linkers.

[0247] Calculated binding energies between protein-ligand complexes were obtained using the FlexX score (an implementation of the Bohm scoring function) within the Tripos software suite. The form for that equation is shown in the equation below:

$$\Delta G_{bind} = \Delta G_{tr} + \Delta G_{hb} + \Delta G_{ion} + \Delta G_{lipo} + \Delta G_{arom} + \Delta G_{rot}$$

[0248] where: ΔG_{tr} is a constant term that accounts for the overall loss of rotational and translational entropy of the lignand, ΔG_{hb} accounts for hydrogen bonds formed between the ligand and protein, ΔG_{ion} accounts for the ionic interactions between the ligand and protein, ΔG_{lipo} accounts for the lipophilic interaction that corresponds to the protein-ligand contact surface, ΔG_{arom} accounts for interactions between aromatic rings in the protein and ligand, and ΔG_{rot} accounts for the entropic penalty of restricting rotatable bonds in the ligand upon binding.

[0249] This method estimates the free energy that a lead compound should have to a target protein for which there is a crystal structure, and it accounts for the entropic penalty of flexible linkers. It can therefore be used to estimate the free energy penalty incurred by attaching linkers to molecules being screened and the binding energy that a lead compound should have in order to overcome the free energy penalty of the linker. The

method does not account for solvation and the entropic penalty is likely overestimated for cases where the linker is bound to a solid phase through another binding complex, such as a biotin:streptavidin complex.

[0250] Co-crystals were aligned by superimposing residues of PIM-1 with corresponding residues in CDK2. The PIM-1 structure used for these calculations was a co-crystal of PIM-1 with a binding compound. The CDK2:Staurosporine co-crystal used was from the Brookhaven database file 1aq1. Hydrogen atoms were added to the proteins and atomic charges were assigned using the AMBER95 parameters within Sybyl. Modifications to the compounds described were made within the Sybyl modeling suite from Tripos.

[0251] These calcualtions indicate that the calculated binding energy for compounds that bind strongly to a given target (such as Staurosporine:CDK2) can be lower than –25 kcal/mol, while the calculated binding affinity for a good scaffold or an unoptimized binding compound can be in the range of -15 to -20. The free energy penalty for attachment to a linker such as the ethylene glycol or hexatriene is estimated as typically being in the range of +5 to +15 kcal/mol.

Linkers

[0252] Linkers suitable for use in the invention can be of many different types. Linkers can be selected for particular applications based on factors such as linker chemistry compatible for attachment to a binding compound and to another component utilized in the particular application. Additional factors can include, without limitation, linker length, linker stability, and ability to remove the linker at an appropriate time. Exemplary linkers include, but are not limited to, hexyl, hexatrienyl, ethylene glycol, and peptide linkers. Traceless linkers can also be used, *e.g.*, as described in Plunkett, M. J., and Ellman, J. A., (1995), *J. Org. Chem.*, 60:6006.

[0253] Typical functional groups, that are utilized to link binding compound(s), include, but not limited to, carboxylic acid, amine, hydroxyl, and thiol. (Examples can be found in Solid-supported combinatorial and parallel synthesis of small molecular weight compound libraries; (1998) Tetrahedron organic chemistry series Vol.17; Pergamon; p85).

Labels

[0254] As indicated above, labels can also be attached to a binding compound or to a linker attached to a binding compound. Such attachment may be direct (attached directly to the binding compound) or indirect (attached to a component that is directly or indirectly attached to the binding compound). Such labels allow detection of the compound either directly or indirectly. Attachement of labels can be performed using conventional chemistries. Labels can include, for example, fluorescent labels, radiolabels, light scattering particles, light absorbent particles, magnetic particles, enzymes, and specific binding agents (*e.g.*, biotin or an antibody target moiety).

Solid Phase Media

[0255] Additional examples of components that can be attached directly or indirectly to a binding compound include various solid phase media. Similar to attachment of linkers and labels, attachment to solid phase media can be performed using conventional chemistries. Such solid phase media can include, for example, small components such as beads, nanoparticles, and fibers (*e.g.*, in suspension or in a gel or chromatographic matrix). Likewise, solid phase media can include larger objects such as plates, chips, slides, and tubes. In many cases, the binding compound will be attached in only a portion of such an objects, *e.g.*, in a spot or other local element on a generally flat surface or in a well or portion of a well.

Identification of Biological Agents

[0256] The posession of structural information about a protein also provides for the identification of useful biological agents, such as epitpose for development of antibodies, identification of mutation sites expected to affect activity, and identification of attachment sites allowing attachment of the protein to materials such as labels, linkers, peptides, and solid phase media.

[0257] Antibodies (Abs) finds multiple applications in a variety of areas including biotechnology, medicine and diagnosis, and indeed they are one of the most powerful tools for life science research. Abs directed against protein antigens can recognize either linear or native three-dimensional (3D) epitopes. The obtention of Abs that recognize 3D epitopes require the use of whole native protein (or of a portion that assumes a native conformation) as immunogens. Unfortunately, this not always a choice due to various technical reasons: for example the native protein is just not available, the protein is toxic,

or its is desirable to utilize a high density antigen presentation. In such cases, immunization with peptides is the alternative. Of course, Abs generated in this manner will recognize linear epitopes, and they might or might not recognize the source native protein, but yet they will be useful for standard laboratory applications such as western blots. The selection of peptides to use as immunogens can be accomplished by following particular selection rules and/or use of epitope prediction software.

[0258] Though methods to predict antigenic peptides are not infallible, there are several rules that can be followed to determine what peptide fragments from a protein are likely to be antigenic. These rules are also dictated to increase the likelihood that an Ab to a particular peptide will recognize the native protein.

- 1. Antigenic peptides should be located in solvent accessible regions and contain both hydrophobic and hydrophilic residues.
    - For proteins of known 3D structure, solvent accessibility can be determined using a variety of programs such as DSSP, NACESS, or WHATIF, among others.
    - If the 3D structure is not known, use any of the following web servers to predict accessibilities: PHD, JPRED, PredAcc (c) ACCpro
- 2. Preferably select peptides lying in long loops connecting Secondary Structure (SS) motifs, avoiding peptides located in helical regions. This will increase the odds that the Ab recognizes the native protein. Such peptides can, for example, be identified from a crystal structure or crystal structure-based homology model.
    - For protein with known 3D coordinates, SS can be obtained from the sequence link of the relevant entry at the Brookhaven data bank. The PDBsum server also offer SS analysis of pdb records.
    - When no structure is available secondary structure predictions can be obtained from any of the following servers: PHD, JPRED, PSI-PRED, NNSP, etc
- 3. When possible, choose peptides that are in the N- and C-terminal region of the protein. Because the N- and C- terminal regions of proteins are usually solvent

accessible and unstructured, Abs against those regions are also likely to recognize the native protein.

- 4. For cell surface glycoproteins, eliminate from initial peptides those containing consesus sites for N-glycosilation.
    - N-glycosilation sites can be detected using Scanprosite, or NetNGlyc

[0259] In addition, several methods based on various physio-chemical properties of experimental determined epitopes (flexibility, hydrophibility, accessibility) have been published for the prediction of antigenic determinants and can be used. The antigenic index and Preditop are example.

[0260] Perhaps the simplest method for the prediction of antigenic determinants is that of Kolaskar and Tongaonkar, which is based on the occurrence of amino acid residues in experimentally determined epitopes. (Kolaskar and Tongaonkar (1990) A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBBS Lett.* 276(1-2):172-174.) The prediction algorithm works as follows:

- 1. Calculate the average propensity for each overlapping 7-mer and assign the result to the central residue (i+3) of the 7-mer.
- 2. Calculate the average for the whole protein.
- 3. (a) If the average for the whole protein is above 1.0 then all residues having average propensity above 1.0 are potentially antigenic.
- 3. (b) If the average for the whole protein is below 1.0 then all residues having above the average for the whole protein are potentially antigenic.
- 4. Find 8-mers where all residues are selected by step 3 above (6-mers in the original paper)

[0261] The Kolaskar and Tongaonkar method is also available from the GCG package, and it runs using the command *egcg*.

[0262] Crystal structures also allow identification of residues at which mutation is likely to alter the activity of the protein. Such residues include, for example, residues that

interact with susbtrate, conserved active site residues, and residues that are in a region of ordered secondary structure of involved in tertiary interactions. The mutations that are likely to affect activity will vary for different molecular contexts. Mutations in an active site that will affect activity are typically substitutions or deletions that eliminate a charge-charge or hydrogen bonding interaction, or introduce a steric interference. Mutations in secondary structure regions or molecular interaction regions that are likely to affect activity include, for example, substitutions that alter the hydrophobicity/hydrophilicity of a region, or that introduce a sufficient strain in a region near or including the active site so that critical residue(s) in the active site are displaced. Such substitutions and/or deletions and/or insertions are recognized, and the predicted structural and/or energetic effects of mutations can be calculated using conventional software.

IX. Phosphodiesterase Activity Assays

[0263] A number of different assays for phosphodiesterase activity can be utilized for assaying for active modulators and/or determining specificity of a modulator for a particular phosphodiesterase or group or phosphodiesterases. In addition to the assay mentioned in the Examples below, one of ordinary skill in the art will know of other assays that can be utilized and can modify an assay for a particular application. For example, numerous papers concerning PDE5 as well as papers concerning other PDEs described assays that can be used. For example, useful assays are described in Fryburg et al., U.S. Patent Application Publication 2002/0165237, Thompson et al., U.S. Patent Application Publication 2002/0009764, Pamukcu et al., U.S. Patent Application 09/046,739, and Pamukcu et al., U.S. Patent 6,500,610.

[0264] An assay for phosphodiesterase activity that can be used for PDE5A, can be performed according to the following procedure using purified PDE5A using the procedure described in Example 6.

[0265] Additional alternative assays can employ binding determinations. For example, this sort of assay can be formatted either in a fluorescence resonance energy transfer (FRET) format, or using an AlphaScreen (*a*mplified *l*uminescent *p*roximity *h*omogeneous *a*ssay) format by varying the donor and acceptor reagents that are attached to streptavidin or the phosphor-specific antibody.

X. Organic Synthetic Techniques

[0266] The versatility of computer-based modulator design and identification lies in the diversity of structures screened by the computer programs. The computer programs can search databases that contain very large numbers of molecules and can modify modulators already complexed with the enzyme with a wide variety of chemical functional groups. A consequence of this chemical diversity is that a potential modulator of phosphodiesterase function may take a chemical form that is not predictable. A wide array of organic synthetic techniques exist in the art to meet the challenge of constructing these potential modulators. Many of these organic synthetic methods are described in detail in standard reference sources utilized by those skilled in the art. One example of suh a reference is March, 1994, Advanced Organic Chemistry; Reactions, Mechanisms and Structure, New York, McGraw Hill. Thus, the techniques useful to synthesize a potential modulator of phosphodiesterase function identified by computer-based methods are readily available to those skilled in the art of organic chemical synthesis.

XI. Administration

[0267] The methods and compounds will typically be used in therapy for human patients. However, they may also be used to treat similar or identical diseases in other vertebrates such as other primates, sports animals, and pets such as horses, dogs and cats.

[0268] Suitable dosage forms, in part, depend upon the use or the route of administration, for example, oral, transdermal, transmucosal, or by injection (parenteral). Such dosage forms should allow the compound to reach target cells. Other factors are well known in the art, and include considerations such as toxicity and dosage forms that retard the compound or composition from exerting its effects. Techniques and formulations generally may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA, 1990 (hereby incorporated by reference herein).

[0269] Compounds can be formulated as pharmaceutically acceptable salts. Pharmaceutically acceptable salts are non-toxic salts in the amounts and concentrations at which they are administered. The preparation of such salts can facilitate the pharmacological use by altering the physical characteristics of a compound without preventing it from exerting its physiological effect. Useful alterations in physical

properties include lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate administering higher concentrations of the drug.

[0270] Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, chloride, hydrochloride, fumarate, maleate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, cyclohexylsulfamate and quinate. Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, maleic acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, cyclohexylsulfamic acid, fumaric acid, and quinic acid.

[0271] Pharmaceutically acceptable salts also include basic addition salts such as those containing benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminum, calcium, lithium, magnesium, potassium, sodium, ammonium, alkylamine, and zinc, when acidic functional groups, such as carboxylic acid or phenol are present. For example, see Remington's Pharmaceutical Sciences, 19th ed., Mack Publishing Co., Easton, PA, Vol. 2, p. 1457, 1995. Such salts can be prepared using the appropriate corresponding bases.

[0272] Pharmaceutically acceptable salts can be prepared by standard techniques. For example, the free-base form of a compound is dissolved in a suitable solvent, such as an aqueous or aqueous-alcohol in solution containing the appropriate acid and then isolated by evaporating the solution. In another example, a salt is prepared by reacting the free base and acid in an organic solvent.

[0273] The pharmaceutically acceptable salt of the different compounds may be present as a complex. Examples of complexes include 8-chlorotheophylline complex (analogous to, *e.g.*, dimenhydrinate: diphenhydramine 8-chlorotheophylline (1:1) complex; Dramamine) and various cyclodextrin inclusion complexes.

[0274] Carriers or excipients can be used to produce pharmaceutical compositions. The carriers or excipients can be chosen to facilitate administration of the compound. Examples of carriers include calcium carbonate, calcium phosphate, various sugars such as lactose, glucose, or sucrose, or types of starch, cellulose derivatives, gelatin, vegetable

oils, polyethylene glycols and physiologically compatible solvents. Examples of physiologically compatible solvents include sterile solutions of water for injection (WFI), saline solution, and dextrose.

[0275] The compounds can be administered by different routes including intravenous, intraperitoneal, subcutaneous, intramuscular, oral, transmucosal, rectal, or transdermal. Oral administration is preferred. For oral administration, for example, the compounds can be formulated into conventional oral dosage forms such as capsules, tablets, and liquid preparations such as syrups, elixirs, and concentrated drops.

[0276] Pharmaceutical preparations for oral use can be obtained, for example, by combining the active compounds with solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose (CMC), and/or polyvinylpyrrolidone (PVP: povidone). If desired, disintegrating agents may be added, such as the cross—linked polyvinylpyrrolidone, agar, or alginic acid, or a salt thereof such as sodium alginate.

[0277] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain, for example, gum arabic, talc, poly-vinylpyrrolidone, carbopol gel, polyethylene glycol (PEG), and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dye-stuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[0278] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin ("gelcaps"), as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils,

liquid paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

[0279] Alternatively, injection (parenteral administration) may be used, *e.g.*, intramuscular, intravenous, intraperitoneal, and/orsubcutaneous. For injection, the compounds of the invention are formulated in sterile liquid solutions, preferably in physiologically compatible buffers or solutions, such as saline solution, Hank's solution, or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms can also be produced.

[0280] Administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration, for example, may be through nasal sprays or suppositories (rectal or vaginal).

[0281] The amounts of various compound to be administered can be determined by standard procedures taking into account factors such as the compound IC_{50}, the biological half-life of the compound, the age, size, and weight of the patient, and the disorder associated with the patient. The importance of these and other factors are well known to those of ordinary skill in the art. Generally, a dose will be between about 0.01 and 50 mg/kg, preferably 0.1 and 20 mg/kg of the patient being treated. Multiple doses may be used.

Manipulation of PDE5A

[0282] As the full-length coding sequence and amino acid sequence of PDE5A is known, cloning, construction of recombinant hPIM-3, production and purification of recombinant protein, introduction of PDE5A into other organisms, and other molecular biological manipulations of PDE5A are readily performed.

[0283] Techniques for the manipulation of nucleic acids, such as, e.g., subcloning, labeling probes (e.g., random-primer labeling using Klenow polymerase, nick translation,

amplification), sequencing, hybridization and the like are well disclosed in the scientific and patent literature, see, e.g., Sambrook, ed., Molecular Cloning: a Laboratory Manual (2nd ed.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989); Current Protocols in Molecular Biology, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization With Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993). [0100] Nucleic acid sequences can be amplified as necessary for further use using amplification methods, such as PCR, isothermal methods, rolling circle methods, etc., are well known to the skilled artisan. See, e.g., Saiki, "Amplification of Genomic DNA" in PCR Protocols, Innis et al., Eds., Academic Press, San Diego, CA 1990, pp 13-20; Wharam et al., *Nucleic Acids Res.* 2001 Jun 1;29(11):E54-E54; Hafner et al., *Biotechniques* 2001 Apr;30(4):852-6, 858, 860 passim; Zhong et al., *Biotechniques* 2001 Apr;30(4):852-6, 858, 860 passim.

[0284] Nucleic acids, vectors, capsids, polypeptides, and the like can be analyzed and quantified by any of a number of general means well known to those of skill in the art. These include, e.g., analytical biochemical methods such as NMR, spectrophotometry, radiography, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and hyperdiffusion chromatography, various immunological methods, e.g. fluid or gel precipitin reactions, immunodiffusion, immuno-electrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immuno-fluorescent assays, Southern analysis, Northern analysis, dot-blot analysis, gel electrophoresis (e.g., SDS-PAGE), nucleic acid or target or signal amplification methods, radiolabeling, scintillation counting, and affinity chromatography.

[0285] Obtaining and manipulating nucleic acids used to practice the methods of the invention can be performed by cloning from genomic samples, and, if desired, screening and re-cloning inserts isolated or amplified from, e.g., genomic clones or cDNA clones. Sources of nucleic acid used in the methods of the invention include genomic or cDNA libraries contained in, e.g., mammalian artificial chromosomes (MACs), see, e.g., U.S. Patent Nos. 5,721,118; 6,025,155; human artificial chromosomes, see, e.g., Rosenfeld (1997) *Nat. Genet.* 15:333-335; yeast artificial chromosomes (YAC); bacterial artificial

chromosomes (BAC); P1 artificial chromosomes, see, e.g., Woon (1998) *Genomics* 50:306-316; P1-derived vectors (PACs), see, e.g., Kern (1997) *Biotechniques* 23:120-124; cosmids, recombinant viruses, phages or plasmids.

[0286] The nucleic acids of the invention can be operatively linked to a promoter. A promoter can be one motif or an array of nucleic acid control sequences which direct transcription of a nucleic acid. A promoter can include necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter which is active under most environmental and developmental conditions. An "inducible" promoter is a promoter which is under environmental or developmental regulation. A "tissue specific" promoter is active in certain tissue types of an organism, but not in other tissue types from the same organism. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

[0287] The nucleic acids of the invention can also be provided in expression vectors and cloning vehicles, e.g., sequences encoding the polypeptides of the invention. Expression vectors and cloning vehicles of the invention can comprise viral particles, baculovirus, phage, plasmids, phagemids, cosmids, fosmids, bacterial artificial chromosomes, viral DNA (e.g., vaccinia, adenovirus, foul pox virus, pseudorabies and derivatives of SV40), P1-based artificial chromosomes, yeast plasmids, yeast artificial chromosomes, and any other vectors specific for specific hosts of interest (such as bacillus, *Aspergillus* and yeast). Vectors of the invention can include chromosomal, non-chromosomal and synthetic DNA sequences. Large numbers of suitable vectors are known to those of skill in the art, and are commercially available.

[0288] The nucleic acids of the invention can be cloned, if desired, into any of a variety of vectors using routine molecular biological methods; methods for cloning *in vitro* amplified nucleic acids are disclosed, *e.g.*, U.S. Pat. No. 5,426,039. To facilitate cloning of amplified sequences, restriction enzyme sites can be "built into" a PCR primer pair.

Vectors may be introduced into a genome or into the cytoplasm or a nucleus of a cell and expressed by a variety of conventional techniques, well described in the scientific and patent literature. See, e.g., Roberts (1987) *Nature* 328:731; Schneider (1995) *Protein Expr. Purif.* 6435:10; Sambrook, Tijssen or Ausubel. The vectors can be isolated from natural sources, obtained from such sources as ATCC or GenBank libraries, or prepared by synthetic or recombinant methods. For example, the nucleic acids of the invention can be expressed in expression cassettes, vectors or viruses which are stably or transiently expressed in cells (e.g., episomal expression systems). Selection markers can be incorporated into expression cassettes and vectors to confer a selectable phenotype on transformed cells and sequences. For example, selection markers can code for episomal maintenance and replication such that integration into the host genome is not required.

[0289] In one aspect, the nucleic acids of the invention are administered *in vivo* for *in situ* expression of the peptides or polypeptides of the invention. The nucleic acids can be administered as "naked DNA" (see, e.g., U.S. Patent No. 5,580,859) or in the form of an expression vector, e.g., a recombinant virus. The nucleic acids can be administered by any route, including peri- or intra-tumorally, as described below. Vectors administered *in vivo* can be derived from viral genomes, including recombinantly modified enveloped or non-enveloped DNA and RNA viruses, preferably selected from baculoviridiae, parvoviridiae, picornoviridiae, herpesveridiae, poxviridae, adenoviridiae, or picornnaviridiae. Chimeric vectors may also be employed which exploit advantageous merits of each of the parent vector properties (See e.g., Feng (1997) *Nature Biotechnology* 15:866-870). Such viral genomes may be modified by recombinant DNA techniques to include the nucleic acids of the invention; and may be further engineered to be replication deficient, conditionally replicating or replication competent. In alternative aspects, vectors are derived from the adenoviral (e.g., replication incompetent vectors derived from the human adenovirus genome, see, e.g., U.S. Patent Nos. 6,096,718; 6,110,458; 6,113,913; 5,631,236); adeno-associated viral and retroviral genomes. Retroviral vectors can include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof; see, e.g., U.S. Patent Nos. 6,117,681; 6,107,478; 5,658,775; 5,449,614; Buchscher (1992) J. Virol. 66:2731-2739; Johann (1992) *J. Virol.* 66:1635-1640). Adeno-associated virus (AAV)-based vectors can be used to transduce cells with target nucleic acids, e.g., in the *in vitro* production of nucleic acids and peptides, and in *in*

vivo and *ex vivo* gene therapy procedures; see, e.g., U.S. Patent Nos. 6,110,456; 5,474,935; Okada (1996) *Gene Ther.* 3:957-964.

[0290] The present invention also relates to fusion proteins, and nucleic acids encoding them. A polypeptide of the invention can be fused to a heterologous peptide or polypeptide, such as N-terminal identification peptides which impart desired characteristics, such as increased stability or simplified purification. Peptides and polypeptides of the invention can also be synthesized and expressed as fusion proteins with one or more additional domains linked thereto for, e.g., producing a more immunogenic peptide, to more readily isolate a recombinantly synthesized peptide, to identify and isolate antibodies and antibody-expressing B cells, and the like. Detection and purification facilitating domains include, e.g., metal chelating peptides such as polyhistidine tracts and histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor Xa or enterokinase (Invitrogen, San Diego CA) between a purification domain and the motif-comprising peptide or polypeptide to facilitate purification. For example, an expression vector can include an epitope-encoding nucleic acid sequence linked to six histidine residues followed by a thioredoxin and an enterokinase cleavage site (see e.g., Williams (1995) *Biochemistry* 34:1787-1797; Dobeli (1998) *Protein Expr. Purif.* 12:404-414). The histidine residues facilitate detection and purification while the enterokinase cleavage site provides a means for purifying the epitope from the remainder of the fusion protein. In one aspect, a nucleic acid encoding a polypeptide of the invention is assembled in appropriate phase with a leader sequence capable of directing secretion of the translated polypeptide or fragment thereof. Technology pertaining to vectors encoding fusion proteins and application of fusion proteins are well disclosed in the scientific and patent literature, see e.g., Kroll (1993) *DNA Cell. Biol.* 12:441-53.

[0291] The nucleic acids and polypeptides of the invention can be bound to a solid support, e.g., for use in screening and diagnostic methods. Solid supports can include, e.g., membranes (e.g., nitrocellulose or nylon), a microtiter dish (e.g., PVC, polypropylene, or polystyrene), a test tube (glass or plastic), a dip stick (e.g., glass, PVC, polypropylene, polystyrene, latex and the like), a microfuge tube, or a glass, silica, plastic,

metallic or polymer bead or other substrate such as paper. One solid support uses a metal (e.g., cobalt or nickel)-comprising column which binds with specificity to a histidine tag engineered onto a peptide.

[0292] Adhesion of molecules to a solid support can be direct (i.e., the molecule contacts the solid support) or indirect (a "linker" is bound to the support and the molecule of interest binds to this linker). Molecules can be immobilized either covalently (e.g., utilizing single reactive thiol groups of cysteine residues (see, e.g., Colliuod (1993) *Bioconjugate Chem.* 4:528-536) or non-covalently but specifically (e.g., via immobilized antibodies (see, e.g., Schuhmann (1991) *Adv. Mater.* 3:388-391; Lu (1995) *Anal. Chem.* 67:83-87; the biotin/strepavidin system (see, e.g., Iwane (1997) *Biophys. Biochem. Res.* Comm. 230:76-80); metal chelating, e.g., Langmuir-Blodgett films (see, e.g., Ng (1995) *Langmuir* 11:4048-55); metal-chelating self-assembled monolayers (see, e.g., Sigal (1996) *Anal. Chem.* 68:490-497) for binding of polyhistidine fusions.

[0293] Indirect binding can be achieved using a variety of linkers which are commercially available. The reactive ends can be any of a variety of functionalities including, but not limited to: amino reacting ends such as N-hydroxysuccinimide (NHS) active esters, imidoesters, aldehydes, epoxides, sulfonyl halides, isocyanate, isothiocyanate, and nitroaryl halides; and thiol reacting ends such as pyridyl disulfides, maleimides, thiophthalimides, and active halogens. The heterobifunctional crosslinking reagents have two different reactive ends, e.g., an amino-reactive end and a thiol-reactive end, while homobifunctional reagents have two similar reactive ends, e.g., bismaleimidohexane (BMH) which permits the cross-linking of sulfhydryl-containing compounds. The spacer can be of varying length and be aliphatic or aromatic. Examples of commercially available homobifunctional cross-linking reagents include, but are not limited to, the imidoesters such as dimethyl adipimidate dihydrochloride (DMA); dimethyl pimelimidate dihydrochloride (DMP); and dimethyl suberimidate dihydrochloride (DMS). Heterobifunctional reagents include commercially available active halogen-NHS active esters coupling agents such as N-succinimidyl bromoacetate and N-succinimidyl (4-iodoacetyl)aminobenzoate (SIAB) and the sulfosuccinimidyl derivatives such as sulfosuccinimidyl(4-iodoacetyl)aminobenzoate (sulfo-SIAB) (Pierce). Another group of coupling agents is the heterobifunctional and thiol cleavable agents

such as N-succinimidyl 3-(2-pyridyidithio)propionate (SPDP) (Pierce Chemicals, Rockford, IL).

[0294] Antibodies can also be used for binding polypeptides and peptides of the invention to a solid support. This can be done directly by binding peptide-specific antibodies to the column or it can be done by creating fusion protein chimeras comprising motif-containing peptides linked to, e.g., a known epitope (e.g., a tag (e.g., FLAG, myc) or an appropriate immunoglobulin constant domain sequence (an "immunoadhesin," see, e.g., Capon (1989) *Nature* 377:525-531 (1989).

[0295] Nucleic acids or polypeptides of the invention can be immobilized to or applied to an array. Arrays can be used to screen for or monitor libraries of compositions (e.g., small molecules, antibodies, nucleic acids, etc.) for their ability to bind to or modulate the activity of a nucleic acid or a polypeptide of the invention. For example, in one aspect of the invention, a monitored parameter is transcript expression of a gene comprising a nucleic acid of the invention. One or more, or, all the transcripts of a cell can be measured by hybridization of a sample comprising transcripts of the cell, or, nucleic acids representative of or complementary to transcripts of a cell, by hybridization to immobilized nucleic acids on an array, or "biochip." By using an "array" of nucleic acids on a microchip, some or all of the transcripts of a cell can be simultaneously quantified. Alternatively, arrays comprising genomic nucleic acid can also be used to determine the genotype of a newly engineered strain made by the methods of the invention. Polypeptide arrays" can also be used to simultaneously quantify a plurality of proteins.

[0296] The terms "array" or "microarray" or "biochip" or "chip" as used herein is a plurality of target elements, each target element comprising a defined amount of one or more polypeptides (including antibodies) or nucleic acids immobilized onto a defined area of a substrate surface. In practicing the methods of the invention, any known array and/or method of making and using arrays can be incorporated in whole or in part, or variations thereof, as disclosed, for example, in U.S. Patent Nos. 6,277,628; 6,277,489; 6,261,776; 6,258,606; 6,054,270; 6,048,695; 6,045,996; 6,022,963; 6,013,440; 5,965,452; 5,959,098; 5,856,174; 5,830,645; 5,770,456; 5,632,957; 5,556,752; 5,143,854; 5,807,522; 5,800,992; 5,744,305; 5,700,637; 5,556,752; 5,434,049; see also, e.g., WO 99/51773; WO 99/09217; WO 97/46313; WO 96/17958; see also, e.g., Johnston (1998) *Curr. Biol.* 8:R171-R174;

Schummer (1997) *Biotechniques* 23:1087-1092; Kern (1997) *Biotechniques* 23:120-124; Solinas-Toldo (1997) *Genes, Chromosomes & Cancer* 20:399-407; Bowtell (1999) *Nature Genetics Supp.* 21:25-32. See also published U.S. patent applications Nos. 20010018642; 20010019827; 20010016322; 20010014449; 20010014448; 20010012537; 20010008765.

Host Cells and Transformed Cells

[0297] The invention also provides a transformed cell comprising a nucleic acid sequence of the invention, *e.g.*, a sequence encoding a polypeptide of the invention, or a vector of the invention. The host cell may be any of the host cells familiar to those skilled in the art, including prokaryotic cells, eukaryotic cells, such as bacterial cells, fungal cells, yeast cells, mammalian cells, insect cells, or plant cells. Exemplary bacterial cells include *E. coli*, *Streptomyces*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. Exemplary insect cells include *Drosophila* S2 and *Spodoptera* Sf9. Exemplary animal cells include CHO, COS or Bowes melanoma or any mouse or human cell line. The selection of an appropriate host is within the abilities of those skilled in the art.

[0298] Vectors may be introduced into the host cells using any of a variety of techniques, including transformation, transfection, transduction, viral infection, gene guns, or Ti-mediated gene transfer. Particular methods include calcium phosphate transfection, DEAE-Dextran mediated transfection, lipofection, or electroporation.

[0299] Engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the invention. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter may be induced by appropriate means (e.g., temperature shift or chemical induction) and the cells may be cultured for an additional period to allow them to produce the desired polypeptide or fragment thereof.

[0300] Cells can be harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract is retained for further purification. Microbial cells employed for expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known to those skilled in the art. The expressed polypeptide or fragment

can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the polypeptide. If desired, high performance liquid chromatography (HPLC) can be employed for final purification steps.

[0301] Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts and other cell lines capable of expressing proteins from a compatible vector, such as the C127, 3T3, CHO, HeLa and BHK cell lines.

[0302] The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Depending upon the host employed in a recombinant production procedure, the polypeptides produced by host cells containing the vector may be glycosylated or may be non-glycosylated. Polypeptides of the invention may or may not also include an initial methionine amino acid residue.

[0303] Cell-free translation systems can also be employed to produce a polypeptide of the invention. Cell-free translation systems can use mRNAs transcribed from a DNA construct comprising a promoter operably linked to a nucleic acid encoding the polypeptide or fragment thereof. In some aspects, the DNA construct may be linearized prior to conducting an *in vitro* transcription reaction. The transcribed mRNA is then incubated with an appropriate cell-free translation extract, such as a rabbit reticulocyte extract, to produce the desired polypeptide or fragment thereof.

[0304] The expression vectors can contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

[0305] For transient expression in mammalian cells, cDNA encoding a polypeptide of interest may be incorporated into a mammalian expression vector, e.g. pcDNA1, which is available commercially from Invitrogen Corporation (San Diego, Calif., U.S.A.; catalogue

number V490-20). This is a multifunctional 4.2 kb plasmid vector designed for cDNA expression in eukaryotic systems, and cDNA analysis in prokaryotes, incorporated on the vector are the CMV promoter and enhancer, splice segment and polyadenylation signal, an SV40 and Polyoma virus origin of replication, and M13 origin to rescue single strand DNA for sequencing and mutagenesis, Sp6 and T7 RNA promoters for the production of sense and anti-sense RNA transcripts and a Col E1-like high copy plasmid origin. A polylinker is located appropriately downstream of the CMV promoter (and 3' of the T7 promoter).

[0306] The cDNA insert may be first released from the above phagemid incorporated at appropriate restriction sites in the pcDNAI polylinker. Sequencing across the junctions may be performed to confirm proper insert orientation in pcDNAI. The resulting plasmid may then be introduced for transient expression into a selected mammalian cell host, for example, the monkey-derived, fibroblast like cells of the COS-1 lineage (available from the American Type Culture Collection, Rockville, Md. as ATCC CRL 1650).

[0307] For transient expression of the protein-encoding DNA, for example, COS-1 cells may be transfected with approximately 8 μg DNA per 10^6 COS cells, by DEAE-mediated DNA transfection and treated with chloroquine according to the procedures described by Sambrook et al, Molecular Cloning: A Laboratory Manual, 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor N.Y, pp. 16.30-16.37. An exemplary method is as follows. Briefly, COS-1 cells are plated at a density of 5 x 10^6 cells/dish and then grown for 24 hours in FBS-supplemented DMEM/F12 medium. Medium is then removed and cells are washed in PBS and then in medium. A transfection solution containing DEAE dextran (0.4 mg/ml), 100 μM chloroquine, 10% NuSerum, DNA (0.4 mg/ml) in DMEM/F12 medium is then applied on the cells 10 ml volume. After incubation for 3 hours at 37 °C, cells are washed in PBS and medium as just described and then shocked for 1 minute with 10% DMSO in DMEM/F12 medium. Cells are allowed to grow for 2-3 days in 10% FBS-supplemented medium, and at the end of incubation dishes are placed on ice, washed with ice cold PBS and then removed by scraping. Cells are then harvested by centrifugation at 1000 rpm for 10 minutes and the cellular pellet is frozen in liquid nitrogen, for subsequent use in protein expression. Northern blot analysis of a thawed aliquot of frozen cells may be used to confirm expression of receptor-encoding cDNA in cells under storage.

[0308] In a like manner, stably transfected cell lines can also prepared, for example, using two different cell types as host: CHO K1 and CHO Pro5. To construct these cell lines, cDNA coding for the relevant protein may be incorporated into the mammalian expression vector pRC/CMV (Invitrogen), which enables stable expression. Insertion at this site places the cDNA under the expression control of the cytomegalovirus promoter and upstream of the polyadenylation site and terminator of the bovine growth hormone gene, and into a vector background comprising the neomycin resistance gene (driven by the SV40 early promoter) as selectable marker.

[0309] An exemplary protocol to introduce plasmids constructed as described above is as follows. The host CHO cells are first seeded at a density of $5x10^5$ in 10% FBS-supplemented MEM medium. After growth for 24 hours, fresh medium is added to the plates and three hours later, the cells are transfected using the calcium phosphate-DNA co-precipitation procedure (Sambrook et al, supra). Briefly, 3 μg of DNA is mixed and incubated with buffered calcium solution for 10 minutes at room temperature. An equal volume of buffered phosphate solution is added and the suspension is incubated for 15 minutes at room temperature. Next, the incubated suspension is applied to the cells for 4 hours, removed and cells were shocked with medium containing 15% glycerol. Three minutes later, cells are washed with medium and incubated for 24 hours at normal growth conditions. Cells resistant to neomycin are selected in 10% FBS-supplemented alpha-MEM medium containing G418 (1 mg/ml). Individual colonies of G418-resistant cells are isolated about 2-3 weeks later, clonally selected and then propagated for assay purposes.

EXAMPLES

[0310] A number of examples involved in the present invention are described below. In most cases, alternative techniques could also be used. For example, techniques, methods, and other information described in Whitaker et al., U.S. Patent Application 2001/0053780 can be used in the present invention. Such techniques and information include, without limitation, cloning, culturing, purification, assaying, screening, use of modulators, sequence information, and information concerning biological role of PDE5A. Each of these references is incorporated by reference herein in its entirety, including drawings.

EXAMPLE 1: Cloning of PDE5A Phosphodiesterase Domain

[0311] PDE5A cDNA sequence was amplified from a Human Kidney QUICK-Clone cDNA library (Clontech, #7112-1) by PCR using the following primers:

PDE5A-S: 5'-GTCGTAT CATATG TCAGCAGCAGAGGAAGAAAC-3' 33 mer
PDE5A-A: 5'-TCTGCA GTCGAC AGGCCACTCAGTTCCGCTTG-3' 32 mer

[0312] The resulting PCR fragment was digested with NdeI and SalI and subcloned into the pET15S vector (shown below). In this expression plasmid, residues 531-875 of PDE5A are in frame with an N-terminal His-tag followed by a thrombin cleavage site.

[0313] The sequence of pET15S, with multi-cloning site is shown below:

T7 promoter

AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCC

RBS

TCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACC

NdeI

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGC**CATATG**GGATCCGG

M G S S H H H H H H S S G L V P R G S H M --------

StuI **SalI**

AATTCAAAGGCCTAC**GTCGAC**TAGAGCCTGCAGT*CTCGAC*CATCATCATCATCATCATTAATAAAAGG[redacted]

---------- *

SpeI **BamHI**

[redacted]GGCCGTT**ACTAGT**GGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGG

IVEX-3 Primer

Bpu1102 I **T7 terminator**

CTGCTGCCACC[redacted]ACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG

3'-PET Primer

[0314] pET15S vector is derived from pET15b vector (Novagen) for bacterial expression to produce the proteins with N-terminal His6. This vector was modified by

replacement of NdeI-BamHI fragment to others to create a SalI site and stop codon (TAG). Vector size is 5814 bp. Insertion can be performed using NdeI-SalI site.

[0315] The nucleotide and amino acid sequences for the PDE5A phosphodiesterase domain utilized encompass amino acids 531-875 of the amino acid sequence provided in Table 4.

EXAMPLE 2: Expression and Purification of PDE5A Phosphodiesterase Domain

[0316] PDE 5A is purified from *E. coli* cells [BL21(DE3)Codon Plus(RIL) (Novagen)] grown in Terrific broth that has been supplemented with 0.2mM Zinc Acetate and 1mM MgCl2 and induced for 16-20h with 1 mM IPTG at 22 C. The centrifuged bacterial pellet (typically 200-250g from 16 L) is suspended in lysis buffer (0.1M potassium phosphate buffer, pH 8.0, 10% glycerol, 1 mM PMSF). 100ug/ml of lysozyme is added to the lysate and the cells are lysed in a Cell Disruptor (MircoFluidics). The cell extract is clarified at 5000 rpm in a Sorvall SA6000 rotor for 1h, and the supernatant is recentrifuged for another hour at 17000 rpm in a Sorvall SA 600 rotor. 5 mM imidazole (pH 8.0) is added to the clarified supernatant and 2 ml of cobalt beads (50% slurry) is added to each 35 ml of extract. The beads are mixed at 4 C for 3-4 h on a Nutator and the beads are recovered by centrifugation at 4000 rpm for 3 min. The pelleted beads are washed several times with lysis buffer and the beads are packed on a BioRad disposable column. The bound protein is eluted with 3-4 column volumes of 0.1M imidazole followed by 0.25M imidazole, both prepared in lysis buffer. The protein eluted from the cobalt beads is concentrated on Centriprep-10 membranes (Amicon) and separated on a Pharmacia Superdex 200 column (26/60) in low salt buffer (25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 14 mM beta-mercaptoethanol). The uncleaved PDE5A is purified by hydroxyapatite chromatography eluted with a phosphate gradient. A final buffer exchange is done on a Pharmacia Superdex 200 column (26/60) in 25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 14 mM beta-mercaptoethanol.

Example 3: Crystallization of PDE5A Phosphodiesterase Domain

[0317] Crystals of purified PDE5 were grown in 10% (w/v) PEG3000, 100 mM phosphate-citrate (pH 4.3), 200 mM NaCl, 1mM DTT, 1mM Sp-cAMP and 8 mg/ml

protein at 4°C, using an Intelliplate (Robbins Scientific, Hampton) by mixing one microliter of protein with one microliter of precipitant, also at 4°C.

Example 4: Diffraction Analysis of PDE5A

[0318] Synchrotron X-ray data for PDE5A was collected at beamline 8.3.1 of the Advanced Light Source (ALS, Lawrence Berkeley National Laboratory, Berkeley) on a Quantum 210 charge-coupled device detector ($\lambda = 1.10$Å). The data were processed using Mosflm () and scaled and reduced with Scala () in CCP4 (). The data processing process was driven by the ELVES automation scripts.

[0319] A ribbon diagram of the PDE5A catalytic domain is shown in Figure 1. Atomic coordinates for the apo protein are provided in Table 1.

Example 5: PDE5A Binding Assays

[0320] Binding assays can be performed in a variety of ways, including a variety of ways known in the art. For example, as indicated above, binding assays can be performed using fluorescence resonance energy transfer (FRET) format, or using an AlphaScreen

[0321] Alternatively, any method which can measure binding of a ligand to the cGMP-binding site can be used. For example, a fluorescent ligand can be used. When bound to PDE5A, the emitted fluorescence is polarized. Once displaced by inhibitor binding, the polarization decreases.

[0322] Determination of IC50 for compounds by competitive binding assays. (Note that K_I is the dissociation constant for inhibitor binding; K_D is the dissociation constant for substrate binding.) For this system, the IC50, inhibitor binding constant and substrate binding constant can be interrelated according to the following formula:

[0323] When using radiolabeled substrate $K_I = \frac{IC50}{1 + [L^*]/K_D}$,

[0324] the IC50 ~ K_I when there is a small amount of labeled substrate.

Example 6: PDE5A Activity Assay

[0325] As an exemplary phosphodiesterase assay, the effect of potential modulators phosphodiesterase activity of PDE5A and other PDEs was measured in the following assay format:

Reagents

Assay Buffer

50 mM Tris, 7.5
8.3 mM $MgCl_2$
1.7 mM EGTA
0.01% BSA

Store @ 4 degrees

RNA binding YSi SPA beads

Beads are 100 mg/ml in water. Dilute to 5 mg/ml in 18 mM Zn using 1M ZnAcetate/$ZnSO_4$ solution(3:1) and water. Store @ 4 degrees.

Low control compounds	**Concentration of 20X DMSO Stock**
PDE1B: 8-methoxymethyl IBMX	20 mM
PDE2A: EHNA	10 mM
PDE3B: Milrinone	2 mM
PDE4D: Rolipram	10 mM
PDE5A: Zaprinast	10 mM
PDE7B: IBMX	40 mM
PDE10A: Dipyridamole	4 mM

Enzyme concentrations (2X final concentration. Diluted in assay buffer)

PDE1B 50 ng/ml
PDE2A 50 ng/ml
PDE3B 10 ng/ml

PDE4D 5 ng/ml

PDE5A 20 ng/ml

PDE7B 25 ng/ml

PDE10A 5 ng/ml)

Radioligands

[^{3}H] cAMP (Amersham TRK559). Dilute 2000X in assay buffer.

[^{3}H] cGMP (Amersham TRK392). For PDE5A assay only. Dilute 2000X in assay buffer.

Protocol

- Make assay plates from 2mM, 96 well master plates by transferring 1ul of
- compound to 384 well plate using BiomekFx. Final concentration of compounds will be ~100 μM. Duplicate assay plates are prepared from each master plate so that compounds are assayed in duplicate.
- To column 23 of the assay plate add 1ul of 20X DMSO stock of appropriate control compound. These will be the low controls.
- Columns 1 and 2 of Chembridge library assay plates and columns 21 and 22 of the Maybridge library assay plates have 1ul DMSO. These are the high controls.
- Using BiomekFx, pipet 10 μl of radioligand into each assay well, then, using the same tips, pipet 10 μl of enzyme into each well.
- Seal assay plate with transparent cover. Centrifuge briefly @ 1000 RPM, them mix on plate shaker for 10 s.
- Incubate @ 30° for 30 min.
- Using BiomekFx, add 10 μl of bead mixture to each assay well. Mix beads thoroughly in reservoir immediately prior to each assay plate addition.
- Re-seal plate with fresh transparent cover. Mix on plate shaker for 10 s, then centrifuge for 1 min. @ 1000 RPM.
- Place plates in counting racks. Let stand for ≥ 30 min, then count on Wallac TriLux using program 8.
- Analyze data as % inhibition of enzyme activity. Average of high controls = 0% inhibition. Average of low controls = 100% inhibition.

Example 9: Site-directed Mutagenesis of PDE5A

[0326] Mutagenesis of PDE5A can be carried out according to the following procedure as described in Molecular Biology: Current Innovations and Future Trends. Eds. A.M. Griffin and H.G.Griffin. (1995) ISBN 1-898486-01-8, Horizon Scientific Press, PO Box 1, Wymondham, Norfolk, U.K., among others.

[0327] In vitro site-directed mutagenesis is an invaluable technique for studying protein structure-function relationships, gene expression and vector modification. Several methods have appeared in the literature, but many of these methods require single-stranded DNA as the template. The reason for this, historically, has been the need for separating the complementary strands to prevent reannealing. Use of PCR in site-directed mutagenesis accomplishes strand separation by using a denaturing step to separate the complementing strands and allowing efficient polymerization of the PCR primers. PCR site-directed methods thus allow site-specific mutations to be incorporated in virtually any double-stranded plasmid; eliminating the need for M13-based vectors or single-stranded rescue.

[0328] It is often desirable to reduce the number of cycles during PCR when performing PCR-based site-directed mutagenesis to prevent clonal expansion of any (undesired) second-site mutations. Limited cycling which would result in reduced product yield, is offset by increasing the starting template concentration. A selection is used to reduce the number of parental molecules coming through the reaction. Also, in order to use a single PCR primer set, it is desirable to optimize the long PCR method. Further, because of the extendase activity of some thermostable polymerases it is often necessary to incorporate an end-polishing step into the procedure prior to end-to-end ligation of the PCR-generated product containing the incorporated mutations in one or both PCR primers.

[0329] The following protocol provides a facile method for site-directed mutagenesis and accomplishes the above desired features by the incorporation of the following steps: (i) increasing template concentration approximately 1000-fold over conventional PCR conditions; (ii) reducing the number of cycles from 25-30 to 5-10; (iii) adding the restriction endonuclease DpnI (recognition target sequence: 5-Gm6ATC-3, where the A residue is methylated) to select against parental DNA (note: DNA isolated from almost all common strains of E. coli is Dam-methylated at the sequence 5-GATC-3); (iv) using Taq Extender in the PCR mix for increased reliability for PCR to 10 kb; (v) using Pfu DNA

polymerase to polish the ends of the PCR product, and (vi) efficient intramolecular ligation in the presence of T4 DNA ligase.

[0330] Plasmid template DNA (approximately 0.5 pmole) is added to a PCR cocktail containing, in 25 ul of 1x mutagenesis buffer: (20 mM Tris HCl, pH 7.5; 8 mM MgCl2; 40 ug/ml BSA); 12-20 pmole of each primer (one of which must contain a 5-prime phosphate), 250 uM each dNTP, 2.5 U Taq DNA polymerase, 2.5 U of Taq Extender (Stratagene).

[0331] The PCR cycling parameters are 1 cycle of: 4 min at 94 C, 2 min at 50 C and 2 min at 72 C; followed by 5-10 cycles of 1 min at 94 C, 2 min at 54 C and 1 min at 72 C (step 1).

[0332] The parental template DNA and the linear, mutagenesis-primer incorporating newly synthesized DNA are treated with DpnI (10 U) and Pfu DNA polymerase (2.5U). This results in the DpnI digestion of the in vivo methylated parental template and hybrid DNA and the removal, by Pfu DNA polymerase, of the Taq DNA polymerase-extended base(s) on the linear PCR product.

[0333] The reaction is incubated at 37 C for 30 min and then transferred to 72 C for an additional 30 min (step 2).

[0334] Mutagenesis buffer (1x, 115 ul, containing 0.5 mM ATP) is added to the DpnI-digested, Pfu DNA polymerase-polished PCR products.

[0335] The solution is mixed and 10 ul is removed to a new microfuge tube and T4 DNA ligase (2-4 U) added.

[0336] The ligation is incubated for greater than 60 min at 37 C (step 3).

[0337] The treated solution is transformed into competent E. coli (step 4).

[0338] In addition to the PCR-based site-directed mutagenesis described above, other methods are available. Examples include those described in Kunkel (1985) Proc. Natl. Acad. Sci. 82:488-492; Eckstein et al. (1985) Nucl. Acids Res. 13:8764-8785; and using the GeneEditor™ Site-Directed Mutageneis Sytem from Promega.

[0339] All patents and other references cited in the specification are indicative of the level of skill of those skilled in the art to which the invention pertains, and are incorporated by reference in their entireties, including any tables and figures, to the same extent as if each reference had been incorporated by reference in its entirety individually.

[0340] One skilled in the art would readily appreciate that the present invention is well adapted to obtain the ends and advantages mentioned, as well as those inherent therein. The methods, variances, and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

[0341] It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. For example, variations can be made to crystallization or co-crystallization conditions for PDE5A proteins and/or various phosphodiesterase domain sequences can be used. Thus, such additional embodiments are within the scope of the present invention and the following claims.

[0342] The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0343] In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

[0344] Also, unless indicated to the contrary, where various numerical values are provided for embodiments, additional embodiments are described by taking any 2 different specified values as the endpoints of a range. Such ranges are also within the scope of the present described invention.

[0345] Thus, additional embodiments are within the scope of the invention and within the following claims.

Table 1

```
HEADER    ----                        XX-XXX-XX   xxxx
COMPND    ---
REMARK   3
REMARK   3 REFINEMENT.
REMARK   3   PROGRAM     : REFMAC 5.1.25
REMARK   3   AUTHORS     : MURSHUDOV,VAGIN,DODSON
REMARK   3
REMARK   3    REFINEMENT TARGET : MAXIMUM LIKELIHOOD
REMARK   3
REMARK   3  DATA USED IN REFINEMENT.
REMARK   3   RESOLUTION RANGE HIGH (ANGSTROMS) :   2.10
REMARK   3   RESOLUTION RANGE LOW  (ANGSTROMS) :  84.51
REMARK   3   DATA CUTOFF            (SIGMA(F)) : NONE
REMARK   3   COMPLETENESS FOR RANGE        (%) :  99.35
REMARK   3   NUMBER OF REFLECTIONS             :   23081
REMARK   3
REMARK   3  FIT TO DATA USED IN REFINEMENT.
REMARK   3   CROSS-VALIDATION METHOD           : THROUGHOUT
REMARK   3   FREE R VALUE TEST SET SELECTION   : RANDOM
REMARK   3   R VALUE     (WORKING + TEST SET) : 0.20593
REMARK   3   R VALUE            (WORKING SET) :  0.20404
REMARK   3   FREE R VALUE                      :  0.24234
REMARK   3   FREE R VALUE TEST SET SIZE   (%) :  5.0
REMARK   3   FREE R VALUE TEST SET COUNT      :  1227
REMARK   3
REMARK   3  FIT IN THE HIGHEST RESOLUTION BIN.
REMARK   3   TOTAL NUMBER OF BINS USED           :      20
REMARK   3   BIN RESOLUTION RANGE HIGH           :    2.100
REMARK   3   BIN RESOLUTION RANGE LOW            :    2.155
REMARK   3   REFLECTION IN BIN     (WORKING SET) :     1696
REMARK   3   BIN R VALUE           (WORKING SET) :    0.296
REMARK   3   BIN FREE R VALUE SET COUNT          :       84
REMARK   3   BIN FREE R VALUE                    :    0.336
REMARK   3
REMARK   3  NUMBER OF NON-HYDROGEN ATOMS USED IN REFINEMENT.
REMARK   3   ALL ATOMS                 :     2555
REMARK   3
REMARK   3  B VALUES.
REMARK   3   FROM WILSON PLOT           (A**2) : NULL
REMARK   3   MEAN B VALUE      (OVERALL, A**2) :  32.944
REMARK   3   OVERALL ANISOTROPIC B VALUE.
REMARK   3    B11 (A**2) :    -1.34
REMARK   3    B22 (A**2) :    -1.34
REMARK   3    B33 (A**2) :     2.01
REMARK   3    B12 (A**2) :    -0.67
REMARK   3    B13 (A**2) :     0.00
REMARK   3    B23 (A**2) :     0.00
REMARK   3
REMARK   3  ESTIMATED OVERALL COORDINATE ERROR.
REMARK   3   ESU BASED ON R VALUE                                (A):   0.195
REMARK   3   ESU BASED ON FREE R VALUE                           (A):   0.173
REMARK   3   ESU BASED ON MAXIMUM LIKELIHOOD                     (A):   0.131
REMARK   3   ESU FOR B VALUES BASED ON MAXIMUM LIKELIHOOD (A**2):   5.040
REMARK   3
REMARK   3 CORRELATION COEFFICIENTS.
REMARK   3   CORRELATION COEFFICIENT FO-FC      :   0.957
REMARK   3   CORRELATION COEFFICIENT FO-FC FREE :   0.946
REMARK   3
REMARK   3  RMS DEVIATIONS FROM IDEAL VALUES        COUNT    RMS    WEIGHT
REMARK   3   BOND LENGTHS REFINED ATOMS        (A):  2506 ; 0.014 ; 0.021
REMARK   3   BOND LENGTHS OTHERS               (A):  2282 ; 0.002 ; 0.020
REMARK   3   BOND ANGLES REFINED ATOMS   (DEGREES):  3376 ; 1.501 ; 1.953
REMARK   3   BOND ANGLES OTHERS          (DEGREES):  5327 ; 0.947 ; 3.000
```

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REMARK   3   TORSION ANGLES, PERIOD 1    (DEGREES):   297 ; 5.771 ; 5.000
REMARK   3   CHIRAL-CENTER RESTRAINTS       (A**3):   382 ; 0.086 ; 0.200
REMARK   3   GENERAL PLANES REFINED ATOMS      (A):  2718 ; 0.010 ; 0.020
REMARK   3   GENERAL PLANES OTHERS             (A):   489 ; 0.036 ; 0.020
REMARK   3   NON-BONDED CONTACTS REFINED ATOMS (A):   643 ; 0.239 ; 0.200
REMARK   3   NON-BONDED CONTACTS OTHERS        (A):  2492 ; 0.232 ; 0.200
REMARK   3   NON-BONDED TORSION OTHERS         (A):  1437 ; 0.087 ; 0.200
REMARK   3   H-BOND (X...Y) REFINED ATOMS      (A):    72 ; 0.156 ; 0.200
REMARK   3   POTENTIAL METAL-ION REFINED ATOMS (A):     1 ; 0.041 ; 0.200
REMARK   3   SYMMETRY VDW REFINED ATOMS        (A):    40 ; 0.636 ; 0.200
REMARK   3   SYMMETRY VDW OTHERS               (A):    74 ; 0.393 ; 0.200
REMARK   3   SYMMETRY H-BOND REFINED ATOMS     (A):    18 ; 0.558 ; 0.200
REMARK   3
REMARK   3  ISOTROPIC THERMAL FACTOR RESTRAINTS.     COUNT   RMS    WEIGHT
REMARK   3   MAIN-CHAIN BOND REFINED ATOMS  (A**2):  1502 ; 0.616 ; 1.500
REMARK   3   MAIN-CHAIN ANGLE REFINED ATOMS (A**2):  2417 ; 1.182 ; 2.000
REMARK   3   SIDE-CHAIN BOND REFINED ATOMS  (A**2):  1004 ; 1.967 ; 3.000
REMARK   3   SIDE-CHAIN ANGLE REFINED ATOMS (A**2):   959 ; 3.150 ; 4.500
REMARK   3
REMARK   3  NCS RESTRAINTS STATISTICS
REMARK   3   NUMBER OF DIFFERENT NCS GROUPS :   1
REMARK   3
REMARK   3  NCS GROUP NUMBER               :   1
REMARK   3     CHAIN NAMES                    : A B
REMARK   3     NUMBER OF COMPONENTS NCS GROUP :    3
REMARK   3       COMPONENT C  SSSEQI  TO  C   SSSEQI   CODE
REMARK   3           1     A    534       A     657      6
REMARK   3           1     B    534       B     657      6
REMARK   3           2     A    672       A     686      6
REMARK   3           2     B    672       B     686      6
REMARK   3           3     A    687       A     789      6
REMARK   3           3     B    687       B     789      6
REMARK   3                    GROUP CHAIN         COUNT   RMS     WEIGHT
REMARK   3   LOOSE POSITIONAL   1    A    (A):     11 ;  0.14 ;  5.00
REMARK   3   LOOSE THERMAL      1    A (A**2):     11 ;  4.73 ; 10.00
REMARK   3
REMARK   3
REMARK   3  TLS DETAILS
REMARK   3   NUMBER OF TLS GROUPS  :    2
REMARK   3
REMARK   3   TLS GROUP :     1
REMARK   3    NUMBER OF COMPONENTS GROUP :    4
REMARK   3    COMPONENTS        C SSSEQI   TO  C SSSEQI
REMARK   3    RESIDUE RANGE :   A   534        A   657
REMARK   3    RESIDUE RANGE :   A   672        A   686
REMARK   3    RESIDUE RANGE :   A   687        A   789
REMARK   3    RESIDUE RANGE :   A   804        A   862
REMARK   3    ORIGIN FOR THE GROUP (A):  29.9285   0.5264   7.4989
REMARK   3    T TENSOR
REMARK   3      T11:   0.1470 T22:   0.1360
REMARK   3      T33:   0.1011 T12:   0.0029
REMARK   3      T13:   0.0027 T23:  -0.1172
REMARK   3    L TENSOR
REMARK   3      L11:   4.8960 L22:   2.7854
REMARK   3      L33:   1.2544 L12:   0.7354
REMARK   3      L13:  -0.5427 L23:  -0.0242
REMARK   3    S TENSOR
REMARK   3      S11:   0.3148 S12:  -0.0276 S13:   0.1681
REMARK   3      S21:   0.0297 S22:  -0.3301 S23:   0.3850
REMARK   3      S31:  -0.0433 S32:  -0.0292 S33:   0.0152
REMARK   3
REMARK   3   TLS GROUP :     2
REMARK   3    NUMBER OF COMPONENTS GROUP :    1
REMARK   3    COMPONENTS        C SSSEQI   TO  C SSSEQI
REMARK   3    RESIDUE RANGE :   B   686        B   686
REMARK   3    ORIGIN FOR THE GROUP (A):  28.9451 -15.8239   8.3689
REMARK   3    T TENSOR
```

```
REMARK   3      T11:   0.3135 T22:   0.3143
REMARK   3      T33:   0.3134 T12:  -0.0006
REMARK   3      T13:   0.0010 T23:   0.0001
REMARK   3    L TENSOR
REMARK   3      L11:  20.8295 L22:  24.8368
REMARK   3      L33:  60.0722 L12:  -4.3409
REMARK   3      L13: -27.2632 L23:  -5.7801
REMARK   3    S TENSOR
REMARK   3      S11:   0.0102 S12:  -0.2842 S13:   0.2176
REMARK   3      S21:   0.1711 S22:  -0.0107 S23:  -0.6694
REMARK   3      S31:  -0.3272 S32:   1.0664 S33:   0.0006
REMARK   3
REMARK   3
REMARK   3  BULK SOLVENT MODELLING.
REMARK   3   METHOD USED : BABINET MODEL WITH MASK
REMARK   3   PARAMETERS FOR MASK CALCULATION
REMARK   3   VDW PROBE RADIUS   :   1.40
REMARK   3   ION PROBE RADIUS   :   0.80
REMARK   3   SHRINKAGE RADIUS   :   0.80
REMARK   3
REMARK   3  OTHER REFINEMENT REMARKS:
REMARK   3  HYDROGENS HAVE BEEN ADDED IN THE RIDING POSITIONS
REMARK   3
LINK             HIS A 657                    LEU A 672                 gap
LINK             GLN A 789                    LEU A 804                 gap
CRYST1   96.411   96.411   79.026  90.00  90.00 120.00 P 62
SCALE1      0.010372  0.005988  0.000000        0.00000
SCALE2      0.000000  0.011977  0.000000        0.00000
SCALE3      0.000000  0.000000  0.012654        0.00000
ATOM      1  N   GLU A 534      13.637  -6.977  34.115  1.00 46.59           N
ATOM      3  CA  GLU A 534      14.989  -7.244  33.549  1.00 46.71           C
ATOM      5  CB  GLU A 534      15.061  -8.661  32.955  1.00 46.74           C
ATOM      8  CG  GLU A 534      16.480  -9.164  32.666  1.00 46.21           C
ATOM     11  CD  GLU A 534      16.501 -10.377  31.756  1.00 45.94           C
ATOM     12  OE1 GLU A 534      15.606 -11.233  31.869  1.00 48.25           O
ATOM     13  OE2 GLU A 534      17.409 -10.481  30.922  1.00 47.41           O
ATOM     14  C   GLU A 534      15.355  -6.213  32.478  1.00 47.04           C
ATOM     15  O   GLU A 534      14.494  -5.684  31.767  1.00 47.18           O
ATOM     18  N   GLU A 535      16.652  -5.954  32.367  1.00 47.23           N
ATOM     20  CA  GLU A 535      17.198  -5.021  31.392  1.00 47.53           C
ATOM     22  CB  GLU A 535      18.708  -4.862  31.658  1.00 47.84           C
ATOM     25  CG  GLU A 535      19.347  -3.589  31.116  1.00 48.85           C
ATOM     28  CD  GLU A 535      20.293  -3.849  29.958  1.00 51.55           C
ATOM     29  OE1 GLU A 535      19.948  -4.658  29.058  1.00 53.04           O
ATOM     30  OE2 GLU A 535      21.386  -3.234  29.945  1.00 53.75           O
ATOM     31  C   GLU A 535      16.954  -5.432  29.918  1.00 47.41           C
ATOM     32  O   GLU A 535      16.977  -4.580  29.022  1.00 47.72           O
ATOM     33  N   GLU A 536      16.721  -6.720  29.661  1.00 47.05           N
ATOM     35  CA  GLU A 536      16.712  -7.237  28.282  1.00 46.74           C
ATOM     37  CB  GLU A 536      17.271  -8.664  28.272  1.00 47.26           C
ATOM     40  CG  GLU A 536      17.608  -9.202  26.890  1.00 49.14           C
ATOM     43  CD  GLU A 536      18.981  -9.865  26.810  1.00 52.58           C
ATOM     44  OE1 GLU A 536      19.163 -10.733  25.916  1.00 55.06           O
ATOM     45  OE2 GLU A 536      19.884  -9.510  27.615  1.00 53.60           O
ATOM     46  C   GLU A 536      15.320  -7.214  27.641  1.00 45.58           C
ATOM     47  O   GLU A 536      15.148  -6.735  26.517  1.00 45.20           O
ATOM     48  N   THR A 537      14.339  -7.747  28.363  1.00 44.47           N
ATOM     50  CA  THR A 537      12.949  -7.784  27.903  1.00 43.56           C
ATOM     52  CB  THR A 537      12.045  -8.452  28.958  1.00 43.67           C
ATOM     54  OG1 THR A 537      12.382  -7.963  30.264  1.00 44.49           O
ATOM     56  CG2 THR A 537      12.289  -9.955  29.035  1.00 44.07           C
ATOM     60  C   THR A 537      12.388  -6.394  27.619  1.00 42.43           C
ATOM     61  O   THR A 537      11.610  -6.221  26.689  1.00 42.65           O
ATOM     62  N   ARG A 538      12.769  -5.413  28.433  1.00 41.09           N
ATOM     64  CA  ARG A 538      12.226  -4.062  28.303  1.00 40.04           C
ATOM     66  CB  ARG A 538      12.529  -3.221  29.552  1.00 40.12           C
ATOM     69  CG  ARG A 538      11.534  -3.401  30.690  1.00 39.78           C
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ATOM     72  CD  ARG A 538      11.540  -2.271  31.725  1.00 40.15           C
ATOM     75  NE  ARG A 538      11.185  -2.748  33.070  1.00 40.21           N
ATOM     77  CZ  ARG A 538      12.031  -3.339  33.918  1.00 40.09           C
ATOM     78  NH1 ARG A 538      13.302  -3.546  33.583  1.00 40.79           N
ATOM     81  NH2 ARG A 538      11.605  -3.728  35.111  1.00 40.74           N
ATOM     84  C   ARG A 538      12.721  -3.345  27.038  1.00 39.10           C
ATOM     85  O   ARG A 538      11.927  -2.719  26.339  1.00 38.29           O
ATOM     86  N   GLU A 539      14.013  -3.439  26.729  1.00 38.12           N
ATOM     88  CA  GLU A 539      14.513  -2.825  25.510  1.00 37.97           C
ATOM     90  CB  GLU A 539      15.985  -3.171  25.262  1.00 38.18           C
ATOM     93  CG  GLU A 539      16.978  -2.098  25.671  1.00 39.15           C
ATOM     96  CD  GLU A 539      18.404  -2.487  25.300  1.00 40.30           C
ATOM     97  OE1 GLU A 539      18.552  -3.462  24.550  1.00 40.77           O
ATOM     98  OE2 GLU A 539      19.367  -1.846  25.778  1.00 42.40           O
ATOM     99  C   GLU A 539      13.688  -3.284  24.309  1.00 37.73           C
ATOM    100  O   GLU A 539      13.366  -2.489  23.425  1.00 37.24           O
ATOM    101  N   LEU A 540      13.348  -4.573  24.284  1.00 37.69           N
ATOM    103  CA  LEU A 540      12.592  -5.156  23.177  1.00 37.31           C
ATOM    105  CB  LEU A 540      12.498  -6.677  23.339  1.00 37.36           C
ATOM    108  CG  LEU A 540      12.356  -7.576  22.110  1.00 37.64           C
ATOM    110  CD1 LEU A 540      11.465  -8.736  22.453  1.00 37.32           C
ATOM    114  CD2 LEU A 540      11.849  -6.885  20.847  1.00 38.06           C
ATOM    118  C   LEU A 540      11.196  -4.548  23.060  1.00 37.13           C
ATOM    119  O   LEU A 540      10.781  -4.169  21.975  1.00 36.13           O
ATOM    120  N   GLN A 541      10.475  -4.468  24.178  1.00 38.03           N
ATOM    122  CA  GLN A 541       9.124  -3.896  24.200  1.00 38.21           C
ATOM    124  CB  GLN A 541       8.564  -3.886  25.624  1.00 38.94           C
ATOM    127  CG  GLN A 541       8.343  -5.265  26.267  1.00 40.24           C
ATOM    130  CD  GLN A 541       7.319  -6.120  25.543  1.00 41.38           C
ATOM    131  OE1 GLN A 541       6.254  -6.416  26.097  1.00 43.49           O
ATOM    132  NE2 GLN A 541       7.650  -6.550  24.318  1.00 42.15           N
ATOM    135  C   GLN A 541       9.106  -2.471  23.643  1.00 38.18           C
ATOM    136  O   GLN A 541       8.320  -2.163  22.751  1.00 38.20           O
ATOM    137  N   SER A 542       9.977  -1.616  24.176  1.00 38.16           N
ATOM    139  CA  SER A 542      10.171  -0.259  23.658  1.00 38.24           C
ATOM    141  CB  SER A 542      11.413   0.405  24.294  1.00 38.29           C
ATOM    144  OG  SER A 542      11.168   0.706  25.667  1.00 40.50           O
ATOM    146  C   SER A 542      10.346  -0.268  22.146  1.00 37.34           C
ATOM    147  O   SER A 542       9.620   0.397  21.419  1.00 37.32           O
ATOM    148  N   LEU A 543      11.303  -1.050  21.676  1.00 36.77           N
ATOM    150  CA  LEU A 543      11.681  -1.020  20.268  1.00 36.27           C
ATOM    152  CB  LEU A 543      12.926  -1.889  20.028  1.00 35.78           C
ATOM    155  CG  LEU A 543      13.961  -1.395  19.028  1.00 35.13           C
ATOM    157  CD1 LEU A 543      14.656  -2.564  18.401  1.00 35.18           C
ATOM    161  CD2 LEU A 543      13.429  -0.468  17.943  1.00 35.56           C
ATOM    165  C   LEU A 543      10.552  -1.488  19.353  1.00 36.22           C
ATOM    166  O   LEU A 543      10.233  -0.838  18.364  1.00 36.70           O
ATOM    167  N   ALA A 544       9.980  -2.636  19.681  1.00 36.30           N
ATOM    169  CA  ALA A 544       8.928  -3.259  18.885  1.00 36.40           C
ATOM    171  CB  ALA A 544       8.591  -4.635  19.455  1.00 36.48           C
ATOM    175  C   ALA A 544       7.663  -2.416  18.793  1.00 36.41           C
ATOM    176  O   ALA A 544       6.968  -2.451  17.779  1.00 36.62           O
ATOM    177  N   ALA A 545       7.363  -1.662  19.842  1.00 36.50           N
ATOM    179  CA  ALA A 545       6.118  -0.890  19.887  1.00 36.47           C
ATOM    181  CB  ALA A 545       5.498  -0.939  21.290  1.00 36.49           C
ATOM    185  C   ALA A 545       6.352   0.554  19.459  1.00 36.41           C
ATOM    186  O   ALA A 545       5.414   1.316  19.365  1.00 35.99           O
ATOM    187  N   ALA A 546       7.599   0.929  19.164  1.00 36.49           N
ATOM    189  CA  ALA A 546       7.867   2.315  18.792  1.00 36.48           C
ATOM    191  CB  ALA A 546       9.334   2.687  19.042  1.00 36.01           C
ATOM    195  C   ALA A 546       7.480   2.532  17.338  1.00 36.52           C
ATOM    196  O   ALA A 546       7.583   1.618  16.524  1.00 36.69           O
ATOM    197  N   VAL A 547       6.991   3.731  17.037  1.00 36.54           N
ATOM    199  CA  VAL A 547       6.740   4.160  15.667  1.00 36.75           C
ATOM    201  CB  VAL A 547       5.976   5.520  15.623  1.00 37.32           C
ATOM    203  CG1 VAL A 547       5.990   6.140  14.214  1.00 38.11           C
ATOM    207  CG2 VAL A 547       4.547   5.353  16.124  1.00 37.43           C
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ATOM    211  C   VAL A 547       8.089   4.313  14.996  1.00 36.37           C
ATOM    212  O   VAL A 547       8.997   4.931  15.547  1.00 37.11           O
ATOM    213  N   VAL A 548       8.247   3.700  13.830  1.00 35.84           N
ATOM    215  CA  VAL A 548       9.474   3.842  13.059  1.00 34.92           C
ATOM    217  CB  VAL A 548       9.743   2.597  12.203  1.00 35.05           C
ATOM    219  CG1 VAL A 548      11.064   2.729  11.489  1.00 34.26           C
ATOM    223  CG2 VAL A 548       9.721   1.338  13.065  1.00 35.00           C
ATOM    227  C   VAL A 548       9.303   5.069  12.167  1.00 34.49           C
ATOM    228  O   VAL A 548       8.531   5.019  11.219  1.00 34.01           O
ATOM    229  N   PRO A 549       9.976   6.180  12.459  1.00 33.90           N
ATOM    230  CA  PRO A 549       9.824   7.360  11.603  1.00 33.96           C
ATOM    232  CB  PRO A 549      10.636   8.456  12.325  1.00 33.76           C
ATOM    235  CG  PRO A 549      10.862   7.955  13.695  1.00 34.32           C
ATOM    238  CD  PRO A 549      10.901   6.438  13.574  1.00 34.16           C
ATOM    241  C   PRO A 549      10.351   7.104  10.195  1.00 33.88           C
ATOM    242  O   PRO A 549      11.107   6.139   9.961  1.00 34.16           O
ATOM    243  N   SER A 550       9.952   7.968   9.267  1.00 33.64           N
ATOM    245  CA  SER A 550      10.307   7.816   7.871  1.00 34.15           C
ATOM    247  CB  SER A 550       9.515   8.800   7.022  1.00 34.02           C
ATOM    250  OG  SER A 550      10.072  10.094   7.126  1.00 33.86           O
ATOM    252  C   SER A 550      11.807   8.026   7.653  1.00 34.44           C
ATOM    253  O   SER A 550      12.498   8.601   8.480  1.00 35.07           O
ATOM    254  N   ALA A 551      12.309   7.537   6.536  1.00 34.56           N
ATOM    256  CA  ALA A 551      13.690   7.773   6.158  1.00 34.63           C
ATOM    258  CB  ALA A 551      13.983   7.122   4.805  1.00 34.69           C
ATOM    262  C   ALA A 551      14.025   9.262   6.117  1.00 34.98           C
ATOM    263  O   ALA A 551      15.087   9.675   6.604  1.00 34.64           O
ATOM    264  N   GLN A 552      13.134  10.049   5.518  1.00 35.27           N
ATOM    266  CA  GLN A 552      13.305  11.495   5.424  1.00 36.31           C
ATOM    268  CB  GLN A 552      12.108  12.129   4.698  1.00 36.61           C
ATOM    271  CG  GLN A 552      12.240  13.658   4.434  1.00 38.76           C
ATOM    274  CD  GLN A 552      10.971  14.236   3.807  1.00 41.89           C
ATOM    275  OE1 GLN A 552      10.537  13.767   2.751  1.00 43.18           O
ATOM    276  NE2 GLN A 552      10.361  15.230   4.465  1.00 44.04           N
ATOM    279  C   GLN A 552      13.476  12.162   6.800  1.00 36.32           C
ATOM    280  O   GLN A 552      14.398  12.938   6.990  1.00 36.63           O
ATOM    281  N   THR A 553      12.566  11.868   7.725  1.00 36.31           N
ATOM    283  CA  THR A 553      12.619  12.377   9.098  1.00 36.39           C
ATOM    285  CB  THR A 553      11.422  11.841   9.922  1.00 36.22           C
ATOM    287  OG1 THR A 553      10.178  12.197   9.301  1.00 36.41           O
ATOM    289  CG2 THR A 553      11.359  12.516  11.300  1.00 36.57           C
ATOM    293  C   THR A 553      13.916  11.958   9.804  1.00 36.49           C
ATOM    294  O   THR A 553      14.472  12.719  10.589  1.00 36.70           O
ATOM    295  N   LEU A 554      14.390  10.747   9.541  1.00 36.10           N
ATOM    297  CA  LEU A 554      15.578  10.252  10.242  1.00 36.39           C
ATOM    299  CB  LEU A 554      15.532   8.735  10.373  1.00 36.37           C
ATOM    302  CG  LEU A 554      14.432   8.145  11.243  1.00 36.80           C
ATOM    304  CD1 LEU A 554      14.471   6.628  11.089  1.00 37.50           C
ATOM    308  CD2 LEU A 554      14.582   8.566  12.701  1.00 36.05           C
ATOM    312  C   LEU A 554      16.905  10.692   9.604  1.00 36.32           C
ATOM    313  O   LEU A 554      17.956  10.487  10.191  1.00 36.86           O
ATOM    314  N   LYS A 555      16.845  11.311   8.427  1.00 36.57           N
ATOM    316  CA  LYS A 555      18.021  11.840   7.727  1.00 36.89           C
ATOM    318  CB  LYS A 555      18.772  12.862   8.597  1.00 37.14           C
ATOM    321  CG  LYS A 555      17.922  13.972   9.133  1.00 38.89           C
ATOM    324  CD  LYS A 555      18.742  14.950   9.972  1.00 42.02           C
ATOM    327  CE  LYS A 555      17.839  16.017  10.608  1.00 43.95           C
ATOM    330  NZ  LYS A 555      18.583  17.280  11.057  1.00 46.49           N
ATOM    334  C   LYS A 555      19.007  10.769   7.275  1.00 36.71           C
ATOM    335  O   LYS A 555      20.136  11.089   6.959  1.00 37.24           O
ATOM    336  N   ILE A 556      18.592   9.507   7.242  1.00 36.27           N
ATOM    338  CA  ILE A 556      19.500   8.415   6.944  1.00 35.92           C
ATOM    340  CB  ILE A 556      18.930   7.099   7.436  1.00 36.15           C
ATOM    342  CG1 ILE A 556      17.578   6.808   6.762  1.00 35.82           C
ATOM    345  CD1 ILE A 556      17.211   5.371   6.785  1.00 36.65           C
ATOM    349  CG2 ILE A 556      18.808   7.132   8.926  1.00 37.25           C
ATOM    353  C   ILE A 556      19.875   8.267   5.476  1.00 35.83           C
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ATOM    354  O   ILE A 556      20.796   7.523   5.164  1.00 35.57           O
ATOM    355  N   THR A 557      19.145   8.925   4.585  1.00 36.11           N
ATOM    357  CA  THR A 557      19.498   8.982   3.163  1.00 37.13           C
ATOM    359  CB  THR A 557      18.291   9.458   2.315  1.00 37.47           C
ATOM    361  OG1 THR A 557      17.101   8.764   2.713  1.00 38.12           O
ATOM    363  CG2 THR A 557      18.447   9.061   0.838  1.00 38.48           C
ATOM    367  C   THR A 557      20.707   9.891   2.904  1.00 37.31           C
ATOM    368  O   THR A 557      21.322   9.813   1.846  1.00 37.89           O
ATOM    369  N   ASP A 558      21.067  10.717   3.885  1.00 37.34           N
ATOM    371  CA  ASP A 558      22.131  11.705   3.731  1.00 37.90           C
ATOM    373  CB  ASP A 558      21.983  12.865   4.748  1.00 38.69           C
ATOM    376  CG  ASP A 558      20.629  13.574   4.660  1.00 41.60           C
ATOM    377  OD1 ASP A 558      19.745  13.072   3.924  1.00 46.07           O
ATOM    378  OD2 ASP A 558      20.363  14.638   5.284  1.00 44.02           O
ATOM    379  C   ASP A 558      23.501  11.103   3.942  1.00 37.29           C
ATOM    380  O   ASP A 558      23.745  10.454   4.960  1.00 36.55           O
ATOM    381  N   PHE A 559      24.409  11.371   3.009  1.00 36.83           N
ATOM    383  CA  PHE A 559      25.811  11.066   3.216  1.00 37.19           C
ATOM    385  CB  PHE A 559      26.616  11.405   1.961  1.00 37.33           C
ATOM    388  CG  PHE A 559      26.453  10.399   0.852  1.00 36.15           C
ATOM    389  CD1 PHE A 559      25.982  10.791  -0.392  1.00 34.77           C
ATOM    391  CE1 PHE A 559      25.840   9.878  -1.408  1.00 34.14           C
ATOM    393  CZ  PHE A 559      26.139   8.550  -1.191  1.00 32.43           C
ATOM    395  CE2 PHE A 559      26.609   8.131   0.050  1.00 33.75           C
ATOM    397  CD2 PHE A 559      26.759   9.062   1.066  1.00 35.34           C
ATOM    399  C   PHE A 559      26.397  11.784   4.458  1.00 37.80           C
ATOM    400  O   PHE A 559      27.284  11.258   5.139  1.00 38.12           O
ATOM    401  N   SER A 560      25.858  12.947   4.757  1.00 37.70           N
ATOM    403  CA  SER A 560      26.293  13.784   5.879  1.00 39.03           C
ATOM    405  CB  SER A 560      25.835  15.215   5.579  1.00 39.04           C
ATOM    408  OG  SER A 560      26.651  15.717   4.538  1.00 41.01           O
ATOM    410  C   SER A 560      25.807  13.405   7.300  1.00 39.09           C
ATOM    411  O   SER A 560      26.199  14.033   8.285  1.00 39.31           O
ATOM    412  N   PHE A 561      24.947  12.402   7.382  1.00 39.13           N
ATOM    414  CA  PHE A 561      24.382  11.907   8.629  1.00 38.97           C
ATOM    416  CB  PHE A 561      23.823  10.514   8.364  1.00 38.71           C
ATOM    419  CG  PHE A 561      23.201   9.868   9.550  1.00 37.66           C
ATOM    420  CD1 PHE A 561      21.877  10.112   9.864  1.00 36.57           C
ATOM    422  CE1 PHE A 561      21.280   9.486  10.925  1.00 36.89           C
ATOM    424  CZ  PHE A 561      22.004   8.579  11.680  1.00 37.15           C
ATOM    426  CE2 PHE A 561      23.319   8.309  11.380  1.00 35.98           C
ATOM    428  CD2 PHE A 561      23.922   8.947  10.310  1.00 37.17           C
ATOM    430  C   PHE A 561      25.392  11.802   9.755  1.00 39.21           C
ATOM    431  O   PHE A 561      26.518  11.347   9.516  1.00 38.44           O
ATOM    432  N   SER A 562      24.970  12.166  10.975  1.00 39.53           N
ATOM    434  CA  SER A 562      25.845  12.056  12.148  1.00 40.79           C
ATOM    436  CB  SER A 562      25.934  13.376  12.916  1.00 41.03           C
ATOM    439  OG  SER A 562      26.984  13.244  13.855  1.00 40.22           O
ATOM    441  C   SER A 562      25.615  10.895  13.153  1.00 41.03           C
ATOM    442  O   SER A 562      26.440  10.016  13.224  1.00 45.01           O
ATOM    443  N   ASP A 563      24.573  10.864  13.933  1.00 40.48           N
ATOM    445  CA  ASP A 563      24.485   9.895  15.082  1.00 40.78           C
ATOM    447  CB  ASP A 563      24.996   8.476  14.761  1.00 40.79           C
ATOM    450  CG  ASP A 563      26.311   8.095  15.496  1.00 41.91           C
ATOM    451  OD1 ASP A 563      27.385   8.617  15.150  1.00 42.85           O
ATOM    452  OD2 ASP A 563      26.383   7.226  16.396  1.00 42.36           O
ATOM    453  C   ASP A 563      25.019  10.357  16.478  1.00 40.06           C
ATOM    454  O   ASP A 563      24.619   9.825  17.504  1.00 38.82           O
ATOM    455  N   PHE A 564      25.904  11.340  16.513  1.00 39.33           N
ATOM    457  CA  PHE A 564      26.386  11.857  17.795  1.00 39.36           C
ATOM    459  CB  PHE A 564      27.203  13.141  17.603  1.00 39.66           C
ATOM    462  CG  PHE A 564      28.657  12.870  17.381  1.00 43.24           C
ATOM    463  CD1 PHE A 564      29.200  12.874  16.098  1.00 47.67           C
ATOM    465  CE1 PHE A 564      30.563  12.592  15.875  1.00 47.93           C
ATOM    467  CZ  PHE A 564      31.378  12.287  16.947  1.00 48.79           C
ATOM    469  CE2 PHE A 564      30.834  12.254  18.254  1.00 49.45           C
ATOM    471  CD2 PHE A 564      29.478  12.544  18.455  1.00 47.83           C
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ATOM	473	C	PHE	A	564	25.261	12.052	18.811	1.00 37.82	C
ATOM	474	O	PHE	A	564	25.335	11.532	19.915	1.00 38.99	O
ATOM	475	N	GLU	A	565	24.192	12.714	18.402	1.00 35.36	N
ATOM	477	CA	GLU	A	565	23.147	13.112	19.326	1.00 33.95	C
ATOM	479	CB	GLU	A	565	22.502	14.398	18.801	1.00 34.14	C
ATOM	482	CG	GLU	A	565	23.501	15.529	18.592	1.00 34.68	C
ATOM	485	CD	GLU	A	565	24.101	16.014	19.908	1.00 36.90	C
ATOM	486	OE1	GLU	A	565	23.466	16.857	20.627	1.00 35.83	O
ATOM	487	OE2	GLU	A	565	25.209	15.534	20.224	1.00 37.58	O
ATOM	488	C	GLU	A	565	22.088	12.037	19.601	1.00 31.86	C
ATOM	489	O	GLU	A	565	21.295	12.176	20.524	1.00 29.83	O
ATOM	490	N	LEU	A	566	22.100	10.964	18.819	1.00 30.24	N
ATOM	492	CA	LEU	A	566	21.068	9.928	18.904	1.00 29.66	C
ATOM	494	CB	LEU	A	566	20.992	9.104	17.604	1.00 29.51	C
ATOM	497	CG	LEU	A	566	20.779	9.878	16.294	1.00 30.52	C
ATOM	499	CD1	LEU	A	566	20.689	8.857	15.168	1.00 32.77	C
ATOM	503	CD2	LEU	A	566	19.525	10.745	16.304	1.00 32.40	C
ATOM	507	C	LEU	A	566	21.274	8.966	20.059	1.00 28.90	C
ATOM	508	O	LEU	A	566	22.398	8.657	20.420	1.00 29.20	O
ATOM	509	N	SER	A	567	20.171	8.507	20.629	1.00 27.75	N
ATOM	511	CA	SER	A	567	20.153	7.424	21.601	1.00 27.51	C
ATOM	513	CB	SER	A	567	18.820	7.439	22.353	1.00 27.43	C
ATOM	516	OG	SER	A	567	17.737	7.060	21.502	1.00 25.32	O
ATOM	518	C	SER	A	567	20.298	6.072	20.908	1.00 28.05	C
ATOM	519	O	SER	A	567	20.132	5.978	19.682	1.00 28.56	O
ATOM	520	N	ASP	A	568	20.573	5.021	21.674	1.00 28.29	N
ATOM	522	CA	ASP	A	568	20.710	3.684	21.078	1.00 29.26	C
ATOM	524	CB	ASP	A	568	21.188	2.640	22.108	1.00 29.83	C
ATOM	527	CG	ASP	A	568	22.661	2.767	22.434	1.00 29.32	C
ATOM	528	OD1	ASP	A	568	23.368	3.481	21.729	1.00 29.71	O
ATOM	529	OD2	ASP	A	568	23.187	2.210	23.405	1.00 32.67	O
ATOM	530	C	ASP	A	568	19.376	3.227	20.468	1.00 29.47	C
ATOM	531	O	ASP	A	568	19.348	2.608	19.410	1.00 27.71	O
ATOM	532	N	LEU	A	569	18.283	3.536	21.155	1.00 29.32	N
ATOM	534	CA	LEU	A	569	16.958	3.252	20.624	1.00 29.88	C
ATOM	536	CB	LEU	A	569	15.869	3.644	21.619	1.00 29.64	C
ATOM	539	CG	LEU	A	569	14.454	3.796	21.041	1.00 31.25	C
ATOM	541	CD1	LEU	A	569	13.901	2.447	20.638	1.00 32.64	C
ATOM	545	CD2	LEU	A	569	13.527	4.504	22.040	1.00 33.35	C
ATOM	549	C	LEU	A	569	16.738	3.950	19.273	1.00 29.85	C
ATOM	550	O	LEU	A	569	16.241	3.314	18.340	1.00 30.01	O
ATOM	551	N	GLU	A	570	17.098	5.235	19.167	1.00 29.92	N
ATOM	553	CA	GLU	A	570	16.980	5.962	17.894	1.00 30.00	C
ATOM	555	CB	GLU	A	570	17.325	7.451	18.031	1.00 30.17	C
ATOM	558	CG	GLU	A	570	16.156	8.339	18.478	1.00 30.37	C
ATOM	561	CD	GLU	A	570	16.589	9.717	18.960	1.00 32.39	C
ATOM	562	OE1	GLU	A	570	17.695	9.844	19.501	1.00 30.13	O
ATOM	563	OE2	GLU	A	570	15.814	10.692	18.831	1.00 37.62	O
ATOM	564	C	GLU	A	570	17.798	5.296	16.770	1.00 30.28	C
ATOM	565	O	GLU	A	570	17.308	5.200	15.629	1.00 30.35	O
ATOM	566	N	THR	A	571	19.001	4.794	17.080	1.00 29.57	N
ATOM	568	CA	THR	A	571	19.794	4.091	16.066	1.00 29.67	C
ATOM	570	CB	THR	A	571	21.272	3.816	16.506	1.00 29.48	C
ATOM	572	OG1	THR	A	571	21.314	2.935	17.629	1.00 28.30	O
ATOM	574	CG2	THR	A	571	21.957	5.100	16.974	1.00 28.02	C
ATOM	578	C	THR	A	571	19.130	2.784	15.640	1.00 29.33	C
ATOM	579	O	THR	A	571	19.221	2.379	14.466	1.00 30.05	O
ATOM	580	N	ALA	A	572	18.492	2.124	16.590	1.00 29.18	N
ATOM	582	CA	ALA	A	572	17.756	0.891	16.318	1.00 29.80	C
ATOM	584	CB	ALA	A	572	17.308	0.240	17.602	1.00 29.64	C
ATOM	588	C	ALA	A	572	16.567	1.167	15.401	1.00 30.05	C
ATOM	589	O	ALA	A	572	16.316	0.391	14.495	1.00 30.93	O
ATOM	590	N	LEU	A	573	15.869	2.277	15.612	1.00 30.31	N
ATOM	592	CA	LEU	A	573	14.760	2.688	14.741	1.00 31.26	C
ATOM	594	CB	LEU	A	573	13.975	3.869	15.344	1.00 31.12	C
ATOM	597	CG	LEU	A	573	13.214	3.543	16.635	1.00 32.60	C
ATOM	599	CD1	LEU	A	573	12.544	4.815	17.262	1.00 33.72	C

ATOM	603	CD2	LEU	A	573	12.173	2.452	16.404	1.00	31.36	C
ATOM	607	C	LEU	A	573	15.291	3.033	13.339	1.00	31.87	C
ATOM	608	O	LEU	A	573	14.715	2.619	12.325	1.00	31.11	O
ATOM	609	N	CYS	A	574	16.420	3.735	13.286	1.00	31.88	N
ATOM	611	CA	CYS	A	574	17.080	3.994	12.016	1.00	32.11	C
ATOM	613	CB	CYS	A	574	18.368	4.798	12.228	1.00	32.66	C
ATOM	616	SG	CYS	A	574	18.138	6.536	12.617	1.00	33.97	S
ATOM	617	C	CYS	A	574	17.390	2.690	11.277	1.00	32.33	C
ATOM	618	O	CYS	A	574	17.255	2.609	10.047	1.00	31.91	O
ATOM	619	N	THR	A	575	17.804	1.669	12.023	1.00	32.03	N
ATOM	621	CA	THR	A	575	18.196	0.407	11.427	1.00	31.37	C
ATOM	623	CB	THR	A	575	18.929	-0.460	12.452	1.00	31.74	C
ATOM	625	OG1	THR	A	575	20.116	0.231	12.896	1.00	30.30	O
ATOM	627	CG2	THR	A	575	19.419	-1.765	11.804	1.00	32.94	C
ATOM	631	C	THR	A	575	16.979	-0.332	10.864	1.00	31.23	C
ATOM	632	O	THR	A	575	17.053	-0.878	9.783	1.00	30.31	O
ATOM	633	N	ILE	A	576	15.866	-0.317	11.589	1.00	30.87	N
ATOM	635	CA	ILE	A	576	14.598	-0.848	11.083	1.00	31.22	C
ATOM	637	CB	ILE	A	576	13.497	-0.787	12.139	1.00	30.84	C
ATOM	639	CG1	ILE	A	576	13.882	-1.665	13.329	1.00	30.99	C
ATOM	642	CD1	ILE	A	576	12.992	-1.437	14.558	1.00	33.62	C
ATOM	646	CG2	ILE	A	576	12.119	-1.233	11.549	1.00	31.41	C
ATOM	650	C	ILE	A	576	14.168	-0.119	9.813	1.00	31.43	C
ATOM	651	O	ILE	A	576	13.757	-0.759	8.853	1.00	31.90	O
ATOM	652	N	ARG	A	577	14.301	1.194	9.780	1.00	30.79	N
ATOM	654	CA	ARG	A	577	13.964	1.937	8.585	1.00	31.23	C
ATOM	656	CB	ARG	A	577	14.058	3.437	8.821	1.00	31.07	C
ATOM	659	CG	ARG	A	577	13.601	4.319	7.656	1.00	30.28	C
ATOM	662	CD	ARG	A	577	12.315	3.885	6.972	1.00	29.17	C
ATOM	665	NE	ARG	A	577	11.145	4.167	7.796	1.00	29.79	N
ATOM	667	CZ	ARG	A	577	9.928	3.698	7.577	1.00	28.87	C
ATOM	668	NH1	ARG	A	577	9.679	2.875	6.571	1.00	29.13	N
ATOM	671	NH2	ARG	A	577	8.947	4.051	8.388	1.00	28.52	N
ATOM	674	C	ARG	A	577	14.827	1.544	7.378	1.00	32.02	C
ATOM	675	O	ARG	A	577	14.318	1.486	6.255	1.00	31.52	O
ATOM	676	N	MET	A	578	16.117	1.299	7.602	1.00	32.31	N
ATOM	678	CA	MET	A	578	17.036	0.882	6.525	1.00	32.58	C
ATOM	680	CB	MET	A	578	18.449	0.716	7.061	1.00	32.59	C
ATOM	683	CG	MET	A	578	19.157	2.010	7.334	1.00	35.68	C
ATOM	686	SD	MET	A	578	20.767	1.762	8.093	1.00	38.66	S
ATOM	687	CE	MET	A	578	20.727	3.087	9.249	1.00	37.18	C
ATOM	691	C	MET	A	578	16.587	-0.447	5.895	1.00	32.70	C
ATOM	692	O	MET	A	578	16.530	-0.574	4.660	1.00	33.48	O
ATOM	693	N	PHE	A	579	16.270	-1.425	6.739	1.00	32.11	N
ATOM	695	CA	PHE	A	579	15.767	-2.719	6.277	1.00	32.29	C
ATOM	697	CB	PHE	A	579	15.582	-3.708	7.452	1.00	31.89	C
ATOM	700	CG	PHE	A	579	16.839	-4.434	7.859	1.00	31.60	C
ATOM	701	CD1	PHE	A	579	17.675	-3.926	8.835	1.00	33.58	C
ATOM	703	CE1	PHE	A	579	18.841	-4.613	9.215	1.00	32.33	C
ATOM	705	CZ	PHE	A	579	19.162	-5.807	8.612	1.00	31.30	C
ATOM	707	CE2	PHE	A	579	18.321	-6.319	7.656	1.00	31.22	C
ATOM	709	CD2	PHE	A	579	17.178	-5.632	7.281	1.00	31.99	C
ATOM	711	C	PHE	A	579	14.441	-2.553	5.510	1.00	32.24	C
ATOM	712	O	PHE	A	579	14.185	-3.257	4.523	1.00	32.02	O
ATOM	713	N	THR	A	580	13.604	-1.635	5.970	1.00	32.08	N
ATOM	715	CA	THR	A	580	12.278	-1.430	5.416	1.00	32.46	C
ATOM	717	CB	THR	A	580	11.408	-0.621	6.410	1.00	32.61	C
ATOM	719	OG1	THR	A	580	11.337	-1.312	7.668	1.00	31.75	O
ATOM	721	CG2	THR	A	580	9.934	-0.550	5.950	1.00	33.38	C
ATOM	725	C	THR	A	580	12.318	-0.753	4.043	1.00	32.81	C
ATOM	726	O	THR	A	580	11.677	-1.215	3.108	1.00	33.63	O
ATOM	727	N	ASP	A	581	13.080	0.325	3.922	1.00	33.22	N
ATOM	729	CA	ASP	A	581	13.091	1.144	2.710	1.00	33.25	C
ATOM	731	CB	ASP	A	581	13.577	2.557	3.023	1.00	32.63	C
ATOM	734	CG	ASP	A	581	12.445	3.489	3.515	1.00	34.46	C
ATOM	735	OD1	ASP	A	581	11.400	2.992	4.031	1.00	32.82	O
ATOM	736	OD2	ASP	A	581	12.532	4.741	3.404	1.00	32.84	O

ATOM	737	C	ASP	A	581	13.963	0.518	1.615	1.00	33.04	C
ATOM	738	O	ASP	A	581	13.937	0.956	0.482	1.00	32.09	O
ATOM	739	N	LEU	A	582	14.767	-0.477	1.981	1.00	34.07	N
ATOM	741	CA	LEU	A	582	15.502	-1.291	1.016	1.00	34.19	C
ATOM	743	CB	LEU	A	582	16.827	-1.781	1.606	1.00	34.53	C
ATOM	746	CG	LEU	A	582	17.906	-0.693	1.643	1.00	34.93	C
ATOM	748	CD1	LEU	A	582	19.138	-1.108	2.422	1.00	33.63	C
ATOM	752	CD2	LEU	A	582	18.282	-0.297	0.212	1.00	36.11	C
ATOM	756	C	LEU	A	582	14.639	-2.472	0.590	1.00	34.48	C
ATOM	757	O	LEU	A	582	15.118	-3.352	-0.109	1.00	34.23	O
ATOM	758	N	ASN	A	583	13.381	-2.469	1.031	1.00	34.36	N
ATOM	760	CA	ASN	A	583	12.406	-3.546	0.818	1.00	34.99	C
ATOM	762	CB	ASN	A	583	12.008	-3.640	-0.668	1.00	35.09	C
ATOM	765	CG	ASN	A	583	11.132	-2.495	-1.088	1.00	37.78	C
ATOM	766	OD1	ASN	A	583	10.004	-2.356	-0.609	1.00	39.93	O
ATOM	767	ND2	ASN	A	583	11.647	-1.645	-1.962	1.00	41.11	N
ATOM	770	C	ASN	A	583	12.804	-4.914	1.360	1.00	34.32	C
ATOM	771	O	ASN	A	583	12.303	-5.946	0.882	1.00	34.23	O
ATOM	772	N	LEU	A	584	13.710	-4.947	2.331	1.00	33.18	N
ATOM	774	CA	LEU	A	584	14.185	-6.230	2.854	1.00	33.64	C
ATOM	776	CB	LEU	A	584	15.523	-6.099	3.584	1.00	33.24	C
ATOM	779	CG	LEU	A	584	16.669	-5.565	2.722	1.00	32.96	C
ATOM	781	CD1	LEU	A	584	17.913	-5.315	3.557	1.00	31.66	C
ATOM	785	CD2	LEU	A	584	16.954	-6.513	1.562	1.00	32.83	C
ATOM	789	C	LEU	A	584	13.141	-6.937	3.728	1.00	34.16	C
ATOM	790	O	LEU	A	584	12.989	-8.153	3.647	1.00	34.32	O
ATOM	791	N	VAL	A	585	12.410	-6.178	4.527	1.00	34.42	N
ATOM	793	CA	VAL	A	585	11.444	-6.748	5.461	1.00	35.24	C
ATOM	795	CB	VAL	A	585	10.860	-5.665	6.387	1.00	35.15	C
ATOM	797	CG1	VAL	A	585	9.643	-6.188	7.169	1.00	35.52	C
ATOM	801	CG2	VAL	A	585	11.961	-5.140	7.346	1.00	35.21	C
ATOM	805	C	VAL	A	585	10.290	-7.447	4.730	1.00	36.18	C
ATOM	806	O	VAL	A	585	9.806	-8.526	5.149	1.00	35.50	O
ATOM	807	N	GLN	A	586	9.846	-6.823	3.647	1.00	36.68	N
ATOM	809	CA	GLN	A	586	8.700	-7.340	2.930	1.00	37.07	C
ATOM	811	CB	GLN	A	586	7.877	-6.192	2.338	1.00	37.74	C
ATOM	814	CG	GLN	A	586	8.403	-5.499	1.083	1.00	39.21	C
ATOM	817	CD	GLN	A	586	7.248	-4.869	0.298	1.00	42.46	C
ATOM	818	OE1	GLN	A	586	6.256	-5.540	0.002	1.00	41.35	O
ATOM	819	NE2	GLN	A	586	7.362	-3.575	-0.006	1.00	46.17	N
ATOM	822	C	GLN	A	586	9.099	-8.413	1.901	1.00	36.36	C
ATOM	823	O	GLN	A	586	8.424	-9.427	1.791	1.00	37.00	O
ATOM	824	N	ASN	A	587	10.194	-8.206	1.176	1.00	35.61	N
ATOM	826	CA	ASN	A	587	10.638	-9.191	0.176	1.00	35.71	C
ATOM	828	CB	ASN	A	587	11.765	-8.615	-0.702	1.00	35.65	C
ATOM	831	CG	ASN	A	587	11.305	-7.460	-1.590	1.00	36.24	C
ATOM	832	OD1	ASN	A	587	10.134	-7.102	-1.611	1.00	39.75	O
ATOM	833	ND2	ASN	A	587	12.247	-6.871	-2.328	1.00	36.37	N
ATOM	836	C	ASN	A	587	11.114	-10.533	0.786	1.00	35.57	C
ATOM	837	O	ASN	A	587	11.109	-11.579	0.104	1.00	34.41	O
ATOM	838	N	PHE	A	588	11.578	-10.483	2.038	1.00	35.12	N
ATOM	840	CA	PHE	A	588	12.088	-11.661	2.750	1.00	35.20	C
ATOM	842	CB	PHE	A	588	13.535	-11.407	3.179	1.00	34.99	C
ATOM	845	CG	PHE	A	588	14.465	-11.258	2.018	1.00	34.40	C
ATOM	846	CD1	PHE	A	588	15.075	-10.060	1.740	1.00	32.27	C
ATOM	848	CE1	PHE	A	588	15.895	-9.933	0.634	1.00	34.30	C
ATOM	850	CZ	PHE	A	588	16.102	-11.016	-0.210	1.00	34.81	C
ATOM	852	CE2	PHE	A	588	15.473	-12.207	0.045	1.00	34.03	C
ATOM	854	CD2	PHE	A	588	14.652	-12.321	1.145	1.00	34.98	C
ATOM	856	C	PHE	A	588	11.225	-12.084	3.948	1.00	35.69	C
ATOM	857	O	PHE	A	588	11.635	-12.940	4.735	1.00	35.71	O
ATOM	858	N	GLN	A	589	10.045	-11.476	4.079	1.00	36.23	N
ATOM	860	CA	GLN	A	589	9.063	-11.824	5.119	1.00	37.00	C
ATOM	862	CB	GLN	A	589	8.457	-13.211	4.840	1.00	37.03	C
ATOM	865	CG	GLN	A	589	7.561	-13.284	3.632	1.00	38.23	C
ATOM	868	CD	GLN	A	589	6.518	-14.388	3.787	1.00	41.94	C
ATOM	869	OE1	GLN	A	589	5.522	-14.216	4.506	1.00	45.87	O

ATOM	870	NE2	GLN	A	589	6.749	-15.526	3.139	1.00	41.94	N
ATOM	873	C	GLN	A	589	9.625	-11.791	6.541	1.00	36.91	C
ATOM	874	O	GLN	A	589	9.285	-12.617	7.379	1.00	36.83	O
ATOM	875	N	MET	A	590	10.466	-10.816	6.826	1.00	37.58	N
ATOM	877	CA	MET	A	590	10.983	-10.659	8.180	1.00	37.70	C
ATOM	879	CB	MET	A	590	12.018	-9.572	8.217	1.00	37.85	C
ATOM	882	CG	MET	A	590	13.186	-9.849	7.341	1.00	38.04	C
ATOM	885	SD	MET	A	590	14.419	-8.644	7.693	1.00	35.14	S
ATOM	886	CE	MET	A	590	15.717	-9.257	6.669	1.00	35.41	C
ATOM	890	C	MET	A	590	9.872	-10.288	9.128	1.00	37.86	C
ATOM	891	O	MET	A	590	9.052	-9.443	8.813	1.00	38.38	O
ATOM	892	N	LYS	A	591	9.837	-10.945	10.279	1.00	38.15	N
ATOM	894	CA	LYS	A	591	8.843	-10.663	11.296	1.00	38.30	C
ATOM	896	CB	LYS	A	591	8.629	-11.889	12.170	1.00	38.99	C
ATOM	899	CG	LYS	A	591	7.890	-13.003	11.411	1.00	41.53	C
ATOM	902	CD	LYS	A	591	7.746	-14.298	12.210	1.00	44.02	C
ATOM	905	CE	LYS	A	591	7.783	-15.538	11.299	1.00	45.54	C
ATOM	908	NZ	LYS	A	591	7.982	-16.789	12.087	1.00	46.91	N
ATOM	912	C	LYS	A	591	9.345	-9.496	12.115	1.00	37.85	C
ATOM	913	O	LYS	A	591	10.519	-9.454	12.465	1.00	37.03	O
ATOM	914	N	HIS	A	592	8.463	-8.544	12.402	1.00	37.27	N
ATOM	916	CA	HIS	A	592	8.871	-7.309	13.057	1.00	37.50	C
ATOM	918	CB	HIS	A	592	7.672	-6.412	13.363	1.00	37.66	C
ATOM	921	CG	HIS	A	592	8.051	-5.064	13.905	1.00	38.68	C
ATOM	922	ND1	HIS	A	592	8.522	-4.046	13.101	1.00	40.07	N
ATOM	924	CE1	HIS	A	592	8.765	-2.976	13.842	1.00	40.15	C
ATOM	926	NE2	HIS	A	592	8.478	-3.265	15.100	1.00	39.72	N
ATOM	928	CD2	HIS	A	592	8.041	-4.571	15.168	1.00	40.44	C
ATOM	930	C	HIS	A	592	9.649	-7.558	14.344	1.00	36.99	C
ATOM	931	O	HIS	A	592	10.750	-7.036	14.515	1.00	37.35	O
ATOM	932	N	GLU	A	593	9.082	-8.355	15.239	1.00	36.02	N
ATOM	934	CA	GLU	A	593	9.681	-8.581	16.550	1.00	35.63	C
ATOM	936	CB	GLU	A	593	8.704	-9.334	17.453	1.00	35.97	C
ATOM	939	CG	GLU	A	593	9.338	-9.921	18.702	1.00	37.96	C
ATOM	942	CD	GLU	A	593	8.332	-10.124	19.810	1.00	41.88	C
ATOM	943	OE1	GLU	A	593	7.598	-11.140	19.759	1.00	41.81	O
ATOM	944	OE2	GLU	A	593	8.282	-9.260	20.718	1.00	44.16	O
ATOM	945	C	GLU	A	593	11.018	-9.329	16.471	1.00	34.35	C
ATOM	946	O	GLU	A	593	11.894	-9.161	17.332	1.00	34.16	O
ATOM	947	N	VAL	A	594	11.153	-10.162	15.448	1.00	33.24	N
ATOM	949	CA	VAL	A	594	12.380	-10.908	15.190	1.00	32.19	C
ATOM	951	CB	VAL	A	594	12.153	-12.063	14.144	1.00	31.94	C
ATOM	953	CG1	VAL	A	594	13.463	-12.690	13.701	1.00	32.10	C
ATOM	957	CG2	VAL	A	594	11.245	-13.152	14.729	1.00	31.70	C
ATOM	961	C	VAL	A	594	13.473	-9.950	14.727	1.00	31.67	C
ATOM	962	O	VAL	A	594	14.589	-10.006	15.222	1.00	30.91	O
ATOM	963	N	LEU	A	595	13.139	-9.062	13.796	1.00	31.23	N
ATOM	965	CA	LEU	A	595	14.072	-8.051	13.343	1.00	31.69	C
ATOM	967	CB	LEU	A	595	13.452	-7.192	12.247	1.00	32.15	C
ATOM	970	CG	LEU	A	595	14.354	-6.039	11.779	1.00	32.94	C
ATOM	972	CD1	LEU	A	595	15.661	-6.583	11.252	1.00	34.64	C
ATOM	976	CD2	LEU	A	595	13.628	-5.216	10.716	1.00	33.64	C
ATOM	980	C	LEU	A	595	14.510	-7.159	14.509	1.00	31.38	C
ATOM	981	O	LEU	A	595	15.700	-6.873	14.654	1.00	30.61	O
ATOM	982	N	CYS	A	596	13.551	-6.749	15.338	1.00	30.97	N
ATOM	984	CA	CYS	A	596	13.847	-5.941	16.527	1.00	31.39	C
ATOM	986	CB	CYS	A	596	12.575	-5.557	17.280	1.00	31.32	C
ATOM	989	SG	CYS	A	596	11.599	-4.292	16.461	1.00	34.15	S
ATOM	990	C	CYS	A	596	14.798	-6.652	17.481	1.00	30.86	C
ATOM	991	O	CYS	A	596	15.764	-6.037	17.972	1.00	31.85	O
ATOM	992	N	ARG	A	597	14.542	-7.939	17.716	1.00	30.17	N
ATOM	994	CA	ARG	A	597	15.348	-8.752	18.630	1.00	29.68	C
ATOM	996	CB	ARG	A	597	14.703	-10.111	18.898	1.00	29.75	C
ATOM	999	CG	ARG	A	597	15.395	-10.929	19.982	1.00	30.24	C
ATOM	1002	CD	ARG	A	597	14.737	-12.269	20.280	1.00	31.74	C
ATOM	1005	NE	ARG	A	597	13.494	-12.168	21.053	1.00	32.07	N
ATOM	1007	CZ	ARG	A	597	12.256	-12.327	20.567	1.00	34.88	C

ATOM	1008	NH1	ARG	A	597	12.021	-12.579	19.272	1.00	35.26	N
ATOM	1011	NH2	ARG	A	597	11.223	-12.235	21.392	1.00	36.29	N
ATOM	1014	C	ARG	A	597	16.742	-8.967	18.079	1.00	29.40	C
ATOM	1015	O	ARG	A	597	17.702	-8.986	18.837	1.00	28.15	O
ATOM	1016	N	TRP	A	598	16.835	-9.163	16.764	1.00	29.37	N
ATOM	1018	CA	TRP	A	598	18.120	-9.347	16.095	1.00	28.82	C
ATOM	1020	CB	TRP	A	598	17.928	-9.739	14.621	1.00	29.27	C
ATOM	1023	CG	TRP	A	598	19.248	-9.848	13.909	1.00	28.69	C
ATOM	1024	CD1	TRP	A	598	20.113	-10.900	13.937	1.00	29.65	C
ATOM	1026	NE1	TRP	A	598	21.242	-10.601	13.207	1.00	31.90	N
ATOM	1028	CE2	TRP	A	598	21.118	-9.332	12.699	1.00	29.14	C
ATOM	1029	CD2	TRP	A	598	19.879	-8.827	13.131	1.00	29.44	C
ATOM	1030	CE3	TRP	A	598	19.512	-7.531	12.749	1.00	29.44	C
ATOM	1032	CZ3	TRP	A	598	20.357	-6.821	11.944	1.00	29.79	C
ATOM	1034	CH2	TRP	A	598	21.589	-7.355	11.540	1.00	29.29	C
ATOM	1036	CZ2	TRP	A	598	21.971	-8.609	11.894	1.00	28.46	C
ATOM	1038	C	TRP	A	598	18.979	-8.087	16.215	1.00	28.83	C
ATOM	1039	O	TRP	A	598	20.137	-8.164	16.601	1.00	29.05	O
ATOM	1040	N	ILE	A	599	18.397	-6.929	15.928	1.00	28.80	N
ATOM	1042	CA	ILE	A	599	19.080	-5.646	16.078	1.00	28.57	C
ATOM	1044	CB	ILE	A	599	18.170	-4.467	15.670	1.00	28.39	C
ATOM	1046	CG1	ILE	A	599	17.855	-4.500	14.172	1.00	29.63	C
ATOM	1049	CD1	ILE	A	599	16.764	-3.554	13.747	1.00	30.52	C
ATOM	1053	CG2	ILE	A	599	18.860	-3.137	16.006	1.00	30.68	C
ATOM	1057	C	ILE	A	599	19.602	-5.451	17.495	1.00	28.27	C
ATOM	1058	O	ILE	A	599	20.755	-5.054	17.680	1.00	28.75	O
ATOM	1059	N	LEU	A	600	18.760	-5.742	18.482	1.00	27.81	N
ATOM	1061	CA	LEU	A	600	19.121	-5.596	19.879	1.00	27.28	C
ATOM	1063	CB	LEU	A	600	17.880	-5.666	20.769	1.00	27.30	C
ATOM	1066	CG	LEU	A	600	16.934	-4.451	20.673	1.00	27.61	C
ATOM	1068	CD1	LEU	A	600	15.643	-4.734	21.372	1.00	27.09	C
ATOM	1072	CD2	LEU	A	600	17.568	-3.184	21.253	1.00	28.63	C
ATOM	1076	C	LEU	A	600	20.187	-6.617	20.327	1.00	27.27	C
ATOM	1077	O	LEU	A	600	21.028	-6.290	21.161	1.00	26.24	O
ATOM	1078	N	SER	A	601	20.173	-7.826	19.767	1.00	26.57	N
ATOM	1080	CA	SER	A	601	21.213	-8.837	20.051	1.00	26.67	C
ATOM	1082	CB	SER	A	601	20.821	-10.215	19.479	1.00	26.15	C
ATOM	1085	OG	SER	A	601	19.720	-10.813	20.166	1.00	25.67	O
ATOM	1087	C	SER	A	601	22.573	-8.411	19.453	1.00	26.88	C
ATOM	1088	O	SER	A	601	23.628	-8.653	20.017	1.00	26.05	O
ATOM	1089	N	VAL	A	602	22.549	-7.797	18.283	1.00	27.58	N
ATOM	1091	CA	VAL	A	602	23.780	-7.352	17.656	1.00	27.77	C
ATOM	1093	CB	VAL	A	602	23.508	-6.832	16.231	1.00	27.87	C
ATOM	1095	CG1	VAL	A	602	24.653	-5.965	15.737	1.00	29.12	C
ATOM	1099	CG2	VAL	A	602	23.240	-7.994	15.253	1.00	28.50	C
ATOM	1103	C	VAL	A	602	24.377	-6.242	18.541	1.00	28.34	C
ATOM	1104	O	VAL	A	602	25.548	-6.286	18.926	1.00	27.60	O
ATOM	1105	N	LYS	A	603	23.556	-5.254	18.872	1.00	28.70	N
ATOM	1107	CA	LYS	A	603	24.003	-4.148	19.717	1.00	29.38	C
ATOM	1109	CB	LYS	A	603	22.872	-3.155	19.943	1.00	29.42	C
ATOM	1112	CG	LYS	A	603	23.262	-1.941	20.782	1.00	31.62	C
ATOM	1115	CD	LYS	A	603	22.194	-0.897	20.738	1.00	32.61	C
ATOM	1118	CE	LYS	A	603	21.027	-1.283	21.599	1.00	35.34	C
ATOM	1121	NZ	LYS	A	603	19.869	-0.628	21.043	1.00	39.66	N
ATOM	1125	C	LYS	A	603	24.540	-4.661	21.056	1.00	29.88	C
ATOM	1126	O	LYS	A	603	25.572	-4.211	21.514	1.00	30.29	O
ATOM	1127	N	LYS	A	604	23.850	-5.613	21.671	1.00	30.04	N
ATOM	1129	CA	LYS	A	604	24.324	-6.207	22.923	1.00	30.95	C
ATOM	1131	CB	LYS	A	604	23.352	-7.286	23.424	1.00	30.85	C
ATOM	1134	CG	LYS	A	604	22.126	-6.725	24.009	1.00	35.33	C
ATOM	1137	CD	LYS	A	604	21.072	-7.789	24.380	1.00	38.98	C
ATOM	1140	CE	LYS	A	604	19.936	-7.184	25.234	1.00	40.81	C
ATOM	1143	NZ	LYS	A	604	20.026	-5.712	25.462	1.00	41.19	N
ATOM	1147	C	LYS	A	604	25.708	-6.837	22.784	1.00	30.29	C
ATOM	1148	O	LYS	A	604	26.537	-6.703	23.665	1.00	29.81	O
ATOM	1149	N	ASN	A	605	25.928	-7.572	21.699	1.00	30.06	N
ATOM	1151	CA	ASN	A	605	27.205	-8.263	21.505	1.00	30.33	C

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ATOM   1153  CB  ASN A 605      27.054  -9.347  20.438  1.00 30.19           C
ATOM   1156  CG  ASN A 605      26.464 -10.614  21.027  1.00 31.60           C
ATOM   1157  OD1 ASN A 605      27.171 -11.381  21.662  1.00 34.48           O
ATOM   1158  ND2 ASN A 605      25.154 -10.782  20.910  1.00 29.75           N
ATOM   1161  C   ASN A 605      28.393  -7.351  21.222  1.00 30.31           C
ATOM   1162  O   ASN A 605      29.541  -7.779  21.325  1.00 29.93           O
ATOM   1163  N   TYR A 606      28.096  -6.101  20.864  1.00 30.77           N
ATOM   1165  CA  TYR A 606      29.094  -5.027  20.714  1.00 31.16           C
ATOM   1167  CB  TYR A 606      28.524  -3.899  19.833  1.00 30.63           C
ATOM   1170  CG  TYR A 606      28.757  -4.129  18.378  1.00 31.32           C
ATOM   1171  CD1 TYR A 606      30.042  -4.101  17.865  1.00 31.76           C
ATOM   1173  CE1 TYR A 606      30.287  -4.334  16.537  1.00 32.42           C
ATOM   1175  CZ  TYR A 606      29.238  -4.600  15.683  1.00 31.75           C
ATOM   1176  OH  TYR A 606      29.521  -4.835  14.353  1.00 31.75           O
ATOM   1178  CE2 TYR A 606      27.950  -4.623  16.154  1.00 30.16           C
ATOM   1180  CD2 TYR A 606      27.713  -4.394  17.511  1.00 30.01           C
ATOM   1182  C   TYR A 606      29.553  -4.389  22.023  1.00 31.98           C
ATOM   1183  O   TYR A 606      30.468  -3.594  21.996  1.00 31.56           O
ATOM   1184  N   ARG A 607      28.898  -4.693  23.142  1.00 33.28           N
ATOM   1186  CA  ARG A 607      29.146  -4.016  24.429  1.00 35.02           C
ATOM   1188  CB  ARG A 607      28.336  -4.684  25.551  1.00 35.04           C
ATOM   1191  CG  ARG A 607      26.959  -4.155  25.727  1.00 37.70           C
ATOM   1194  CD  ARG A 607      26.294  -4.678  26.991  1.00 39.36           C
ATOM   1197  NE  ARG A 607      24.844  -4.703  26.835  1.00 42.67           N
ATOM   1199  CZ  ARG A 607      24.010  -5.430  27.581  1.00 43.78           C
ATOM   1200  NH1 ARG A 607      24.466  -6.224  28.552  1.00 43.30           N
ATOM   1203  NH2 ARG A 607      22.700  -5.370  27.345  1.00 45.74           N
ATOM   1206  C   ARG A 607      30.577  -3.987  24.939  1.00 35.46           C
ATOM   1207  O   ARG A 607      31.006  -3.004  25.514  1.00 35.88           O
ATOM   1208  N   LYS A 608      31.290  -5.096  24.824  1.00 36.93           N
ATOM   1210  CA  LYS A 608      32.666  -5.142  25.335  1.00 38.15           C
ATOM   1212  CB  LYS A 608      33.068  -6.578  25.711  1.00 38.79           C
ATOM   1215  CG  LYS A 608      32.304  -7.171  26.889  1.00 39.97           C
ATOM   1218  CD  LYS A 608      33.209  -7.466  28.089  1.00 41.39           C
ATOM   1221  CE  LYS A 608      32.865  -8.784  28.755  1.00 41.54           C
ATOM   1224  NZ  LYS A 608      33.799  -9.043  29.896  1.00 42.55           N
ATOM   1228  C   LYS A 608      33.696  -4.570  24.353  1.00 37.89           C
ATOM   1229  O   LYS A 608      34.874  -4.506  24.682  1.00 38.34           O
ATOM   1230  N   ASN A 609      33.274  -4.159  23.161  1.00 37.47           N
ATOM   1232  CA  ASN A 609      34.227  -3.613  22.187  1.00 37.88           C
ATOM   1234  CB  ASN A 609      33.629  -3.620  20.788  1.00 37.01           C
ATOM   1237  CG  ASN A 609      33.576  -5.002  20.180  1.00 37.42           C
ATOM   1238  OD1 ASN A 609      33.620  -6.011  20.880  1.00 38.35           O
ATOM   1239  ND2 ASN A 609      33.471  -5.053  18.871  1.00 32.99           N
ATOM   1242  C   ASN A 609      34.701  -2.169  22.517  1.00 38.36           C
ATOM   1243  O   ASN A 609      33.946  -1.387  23.070  1.00 36.98           O
ATOM   1244  N   VAL A 610      35.930  -1.824  22.091  1.00 39.12           N
ATOM   1246  CA  VAL A 610      36.442  -0.452  22.196  1.00 39.82           C
ATOM   1248  CB  VAL A 610      37.884  -0.297  21.640  1.00 41.13           C
ATOM   1250  CG1 VAL A 610      38.517   1.049  22.100  1.00 40.50           C
ATOM   1254  CG2 VAL A 610      38.757  -1.484  22.068  1.00 43.89           C
ATOM   1258  C   VAL A 610      35.551   0.386  21.319  1.00 39.18           C
ATOM   1259  O   VAL A 610      34.918  -0.153  20.399  1.00 38.76           O
ATOM   1260  N   ALA A 611      35.527   1.687  21.572  1.00 37.98           N
ATOM   1262  CA  ALA A 611      34.487   2.544  21.029  1.00 37.77           C
ATOM   1264  CB  ALA A 611      34.527   3.959  21.638  1.00 38.26           C
ATOM   1268  C   ALA A 611      34.569   2.609  19.562  1.00 37.90           C
ATOM   1269  O   ALA A 611      33.529   2.668  18.886  1.00 39.09           O
ATOM   1270  N   TYR A 612      35.789   2.555  19.026  1.00 37.10           N
ATOM   1272  CA  TYR A 612      35.907   2.589  17.593  1.00 36.21           C
ATOM   1274  CB  TYR A 612      37.279   3.132  17.144  1.00 35.83           C
ATOM   1277  CG  TYR A 612      38.548   2.537  17.706  1.00 31.94           C
ATOM   1278  CD1 TYR A 612      39.280   3.193  18.678  1.00 30.63           C
ATOM   1280  CE1 TYR A 612      40.486   2.685  19.128  1.00 29.64           C
ATOM   1282  CZ  TYR A 612      40.997   1.524  18.566  1.00 30.05           C
ATOM   1283  OH  TYR A 612      42.199   0.984  18.976  1.00 27.48           O
ATOM   1285  CE2 TYR A 612      40.280   0.859  17.593  1.00 28.47           C
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ATOM	1287	CD2	TYR	A	612	39.090	1.383	17.152	1.00	31.42	C
ATOM	1289	C	TYR	A	612	35.504	1.301	16.870	1.00	36.03	C
ATOM	1290	O	TYR	A	612	35.376	1.316	15.645	1.00	36.29	O
ATOM	1291	N	HIS	A	613	35.285	0.190	17.585	1.00	35.22	N
ATOM	1293	CA	HIS	A	613	34.680	-1.018	16.950	1.00	34.43	C
ATOM	1295	CB	HIS	A	613	35.592	-2.255	17.069	1.00	34.90	C
ATOM	1298	CG	HIS	A	613	36.922	-2.105	16.400	1.00	36.25	C
ATOM	1299	ND1	HIS	A	613	37.063	-1.612	15.113	1.00	35.47	N
ATOM	1301	CE1	HIS	A	613	38.348	-1.602	14.792	1.00	38.87	C
ATOM	1303	NE2	HIS	A	613	39.038	-2.116	15.798	1.00	37.92	N
ATOM	1305	CD2	HIS	A	613	38.168	-2.442	16.818	1.00	37.07	C
ATOM	1307	C	HIS	A	613	33.342	-1.367	17.591	1.00	33.82	C
ATOM	1308	O	HIS	A	613	32.970	-2.551	17.697	1.00	32.40	O
ATOM	1309	N	ASN	A	614	32.651	-0.328	18.049	1.00	33.04	N
ATOM	1311	CA	ASN	A	614	31.435	-0.486	18.822	1.00	32.15	C
ATOM	1313	CB	ASN	A	614	31.384	0.536	19.980	1.00	31.90	C
ATOM	1316	CG	ASN	A	614	31.107	1.966	19.513	1.00	32.57	C
ATOM	1317	OD1	ASN	A	614	30.893	2.227	18.324	1.00	34.59	O
ATOM	1318	ND2	ASN	A	614	31.137	2.895	20.446	1.00	29.51	N
ATOM	1321	C	ASN	A	614	30.202	-0.410	17.902	1.00	31.23	C
ATOM	1322	O	ASN	A	614	30.323	-0.220	16.664	1.00	29.60	O
ATOM	1323	N	TRP	A	615	29.035	-0.579	18.517	1.00	30.30	N
ATOM	1325	CA	TRP	A	615	27.760	-0.531	17.812	1.00	30.70	C
ATOM	1327	CB	TRP	A	615	26.585	-0.716	18.788	1.00	30.92	C
ATOM	1330	CG	TRP	A	615	25.286	-0.217	18.237	1.00	31.99	C
ATOM	1331	CD1	TRP	A	615	24.620	0.880	18.640	1.00	32.83	C
ATOM	1333	NE1	TRP	A	615	23.474	1.035	17.900	1.00	33.36	N
ATOM	1335	CE2	TRP	A	615	23.378	0.012	17.001	1.00	31.11	C
ATOM	1336	CD2	TRP	A	615	24.506	-0.802	17.188	1.00	30.42	C
ATOM	1337	CE3	TRP	A	615	24.647	-1.937	16.381	1.00	32.01	C
ATOM	1339	CZ3	TRP	A	615	23.658	-2.215	15.428	1.00	30.94	C
ATOM	1341	CH2	TRP	A	615	22.556	-1.376	15.269	1.00	31.69	C
ATOM	1343	CZ2	TRP	A	615	22.384	-0.270	16.057	1.00	31.08	C
ATOM	1345	C	TRP	A	615	27.583	0.755	17.002	1.00	30.51	C
ATOM	1346	O	TRP	A	615	27.164	0.694	15.873	1.00	31.12	O
ATOM	1347	N	ARG	A	616	27.963	1.902	17.542	1.00	30.28	N
ATOM	1349	CA	ARG	A	616	27.832	3.144	16.785	1.00	31.43	C
ATOM	1351	CB	ARG	A	616	28.192	4.379	17.621	1.00	31.30	C
ATOM	1354	CG	ARG	A	616	27.202	4.659	18.759	1.00	33.10	C
ATOM	1357	CD	ARG	A	616	25.719	4.547	18.363	1.00	35.82	C
ATOM	1360	NE	ARG	A	616	24.840	4.907	19.468	1.00	37.45	N
ATOM	1362	CZ	ARG	A	616	24.435	6.147	19.764	1.00	37.21	C
ATOM	1363	NH1	ARG	A	616	24.797	7.196	19.031	1.00	35.24	N
ATOM	1366	NH2	ARG	A	616	23.641	6.326	20.806	1.00	36.40	N
ATOM	1369	C	ARG	A	616	28.647	3.113	15.502	1.00	31.51	C
ATOM	1370	O	ARG	A	616	28.148	3.501	14.447	1.00	32.82	O
ATOM	1371	N	HIS	A	617	29.875	2.614	15.546	1.00	31.18	N
ATOM	1373	CA	HIS	A	617	30.606	2.498	14.312	1.00	30.51	C
ATOM	1375	CB	HIS	A	617	32.016	2.029	14.543	1.00	31.02	C
ATOM	1378	CG	HIS	A	617	32.728	1.695	13.269	1.00	30.16	C
ATOM	1379	ND1	HIS	A	617	33.123	2.656	12.380	1.00	27.25	N
ATOM	1381	CE1	HIS	A	617	33.698	2.080	11.344	1.00	31.29	C
ATOM	1383	NE2	HIS	A	617	33.608	0.781	11.493	1.00	28.84	N
ATOM	1385	CD2	HIS	A	617	33.017	0.511	12.696	1.00	29.49	C
ATOM	1387	C	HIS	A	617	29.929	1.577	13.292	1.00	30.71	C
ATOM	1388	O	HIS	A	617	29.861	1.885	12.099	1.00	30.52	O
ATOM	1389	N	ALA	A	618	29.453	0.440	13.750	1.00	30.71	N
ATOM	1391	CA	ALA	A	618	28.829	-0.536	12.861	1.00	30.45	C
ATOM	1393	CB	ALA	A	618	28.498	-1.823	13.630	1.00	30.32	C
ATOM	1397	C	ALA	A	618	27.565	0.061	12.258	1.00	30.85	C
ATOM	1398	O	ALA	A	618	27.290	-0.087	11.071	1.00	31.51	O
ATOM	1399	N	PHE	A	619	26.803	0.755	13.093	1.00	31.16	N
ATOM	1401	CA	PHE	A	619	25.562	1.393	12.682	1.00	30.81	C
ATOM	1403	CB	PHE	A	619	24.853	1.997	13.896	1.00	30.88	C
ATOM	1406	CG	PHE	A	619	23.783	2.974	13.526	1.00	30.88	C
ATOM	1407	CD1	PHE	A	619	22.616	2.546	12.947	1.00	29.68	C
ATOM	1409	CE1	PHE	A	619	21.635	3.459	12.578	1.00	30.57	C

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ATOM   1411  CZ  PHE A 619      21.850   4.791  12.751  1.00 31.51           C
ATOM   1413  CE2 PHE A 619      23.019   5.230  13.319  1.00 32.18           C
ATOM   1415  CD2 PHE A 619      23.986   4.332  13.689  1.00 31.89           C
ATOM   1417  C   PHE A 619      25.849   2.469  11.616  1.00 31.44           C
ATOM   1418  O   PHE A 619      25.139   2.536  10.606  1.00 31.44           O
ATOM   1419  N   ASN A 620      26.896   3.274  11.832  1.00 30.85           N
ATOM   1421  CA  ASN A 620      27.363   4.262  10.836  1.00 31.22           C
ATOM   1423  CB  ASN A 620      28.507   5.126  11.395  1.00 31.15           C
ATOM   1426  CG  ASN A 620      28.010   6.191  12.378  1.00 34.02           C
ATOM   1427  OD1 ASN A 620      27.080   6.934  12.076  1.00 39.28           O
ATOM   1428  ND2 ASN A 620      28.657   6.290  13.543  1.00 35.23           N
ATOM   1431  C   ASN A 620      27.803   3.663   9.498  1.00 31.18           C
ATOM   1432  O   ASN A 620      27.540   4.239   8.454  1.00 31.47           O
ATOM   1433  N   THR A 621      28.484   2.524   9.549  1.00 30.75           N
ATOM   1435  CA  THR A 621      28.868   1.786   8.378  1.00 31.18           C
ATOM   1437  CB  THR A 621      29.655   0.560   8.788  1.00 31.37           C
ATOM   1439  OG1 THR A 621      30.820   0.922   9.574  1.00 33.41           O
ATOM   1441  CG2 THR A 621      30.229  -0.137   7.558  1.00 31.84           C
ATOM   1445  C   THR A 621      27.614   1.362   7.575  1.00 31.48           C
ATOM   1446  O   THR A 621      27.571   1.507   6.374  1.00 30.62           O
ATOM   1447  N   ALA A 622      26.584   0.893   8.266  1.00 32.36           N
ATOM   1449  CA  ALA A 622      25.315   0.494   7.643  1.00 32.22           C
ATOM   1451  CB  ALA A 622      24.433  -0.195   8.662  1.00 32.79           C
ATOM   1455  C   ALA A 622      24.573   1.674   7.064  1.00 32.14           C
ATOM   1456  O   ALA A 622      24.025   1.562   5.973  1.00 31.93           O
ATOM   1457  N   GLN A 623      24.567   2.811   7.769  1.00 31.86           N
ATOM   1459  CA  GLN A 623      23.886   3.999   7.274  1.00 31.61           C
ATOM   1461  CB  GLN A 623      23.809   5.080   8.332  1.00 31.93           C
ATOM   1464  CG  GLN A 623      23.074   6.375   7.924  1.00 31.37           C
ATOM   1467  CD  GLN A 623      23.927   7.312   7.084  1.00 30.66           C
ATOM   1468  OE1 GLN A 623      25.155   7.428   7.290  1.00 31.16           O
ATOM   1469  NE2 GLN A 623      23.293   7.974   6.136  1.00 29.92           N
ATOM   1472  C   GLN A 623      24.554   4.525   6.012  1.00 31.95           C
ATOM   1473  O   GLN A 623      23.859   4.910   5.061  1.00 32.19           O
ATOM   1474  N   CYS A 624      25.884   4.503   5.970  1.00 31.30           N
ATOM   1476  CA  CYS A 624      26.592   4.885   4.759  1.00 31.66           C
ATOM   1478  CB  CYS A 624      28.100   4.862   4.950  1.00 31.87           C
ATOM   1481  SG  CYS A 624      29.005   5.620   3.568  1.00 32.91           S
ATOM   1482  C   CYS A 624      26.217   3.949   3.590  1.00 31.58           C
ATOM   1483  O   CYS A 624      26.085   4.406   2.473  1.00 30.48           O
ATOM   1484  N   MET A 625      26.073   2.653   3.862  1.00 31.89           N
ATOM   1486  CA  MET A 625      25.606   1.680   2.850  1.00 32.02           C
ATOM   1488  CB  MET A 625      25.611   0.262   3.449  1.00 32.34           C
ATOM   1491  CG  MET A 625      25.292  -0.885   2.503  1.00 32.88           C
ATOM   1494  SD  MET A 625      26.447  -1.020   1.189  1.00 35.04           S
ATOM   1495  CE  MET A 625      27.759  -1.743   1.958  1.00 34.33           C
ATOM   1499  C   MET A 625      24.216   2.056   2.326  1.00 32.02           C
ATOM   1500  O   MET A 625      23.997   2.110   1.121  1.00 32.01           O
ATOM   1501  N   PHE A 626      23.283   2.357   3.228  1.00 32.02           N
ATOM   1503  CA  PHE A 626      21.938   2.757   2.838  1.00 31.43           C
ATOM   1505  CB  PHE A 626      21.050   2.992   4.077  1.00 31.75           C
ATOM   1508  CG  PHE A 626      19.652   3.449   3.755  1.00 31.75           C
ATOM   1509  CD1 PHE A 626      18.638   2.523   3.525  1.00 30.61           C
ATOM   1511  CE1 PHE A 626      17.364   2.939   3.231  1.00 32.72           C
ATOM   1513  CZ  PHE A 626      17.066   4.312   3.168  1.00 32.65           C
ATOM   1515  CE2 PHE A 626      18.073   5.235   3.404  1.00 33.06           C
ATOM   1517  CD2 PHE A 626      19.355   4.805   3.689  1.00 31.65           C
ATOM   1519  C   PHE A 626      22.008   4.016   1.987  1.00 31.60           C
ATOM   1520  O   PHE A 626      21.331   4.119   0.960  1.00 31.35           O
ATOM   1521  N   ALA A 627      22.825   4.971   2.409  1.00 31.24           N
ATOM   1523  CA  ALA A 627      22.931   6.228   1.697  1.00 31.47           C
ATOM   1525  CB  ALA A 627      23.790   7.240   2.471  1.00 31.67           C
ATOM   1529  C   ALA A 627      23.520   5.958   0.321  1.00 31.49           C
ATOM   1530  O   ALA A 627      23.007   6.465  -0.679  1.00 31.23           O
ATOM   1531  N   ALA A 628      24.569   5.140   0.266  1.00 30.86           N
ATOM   1533  CA  ALA A 628      25.179   4.800  -1.024  1.00 31.71           C
ATOM   1535  CB  ALA A 628      26.466   4.007  -0.843  1.00 31.46           C
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ATOM   1539  C   ALA A 628      24.193   4.067  -1.955  1.00 31.94           C
ATOM   1540  O   ALA A 628      24.194   4.311  -3.149  1.00 32.27           O
ATOM   1541  N   LEU A 629      23.305   3.243  -1.401  1.00 32.55           N
ATOM   1543  CA  LEU A 629      22.332   2.515  -2.204  1.00 32.89           C
ATOM   1545  CB  LEU A 629      21.719   1.357  -1.403  1.00 33.12           C
ATOM   1548  CG  LEU A 629      22.663   0.204  -1.000  1.00 34.14           C
ATOM   1550  CD1 LEU A 629      22.092  -0.664   0.149  1.00 34.32           C
ATOM   1554  CD2 LEU A 629      22.978  -0.669  -2.144  1.00 34.96           C
ATOM   1558  C   LEU A 629      21.243   3.455  -2.707  1.00 32.75           C
ATOM   1559  O   LEU A 629      20.725   3.285  -3.820  1.00 32.52           O
ATOM   1560  N   LYS A 630      20.895   4.456  -1.897  1.00 32.92           N
ATOM   1562  CA  LYS A 630      19.762   5.340  -2.192  1.00 32.40           C
ATOM   1564  CB  LYS A 630      18.985   5.661  -0.916  1.00 32.67           C
ATOM   1567  CG  LYS A 630      18.220   4.478  -0.352  1.00 34.05           C
ATOM   1570  CD  LYS A 630      17.077   4.068  -1.296  1.00 35.47           C
ATOM   1573  CE  LYS A 630      16.130   3.083  -0.647  1.00 36.74           C
ATOM   1576  NZ  LYS A 630      14.939   2.850  -1.507  1.00 37.93           N
ATOM   1580  C   LYS A 630      20.199   6.626  -2.881  1.00 32.33           C
ATOM   1581  O   LYS A 630      19.934   6.834  -4.055  1.00 32.10           O
ATOM   1582  N   ALA A 631      20.865   7.502  -2.150  1.00 32.35           N
ATOM   1584  CA  ALA A 631      21.341   8.762  -2.723  1.00 31.80           C
ATOM   1586  CB  ALA A 631      21.944   9.623  -1.632  1.00 32.01           C
ATOM   1590  C   ALA A 631      22.385   8.487  -3.810  1.00 31.40           C
ATOM   1591  O   ALA A 631      22.409   9.145  -4.848  1.00 29.37           O
ATOM   1592  N   GLY A 632      23.249   7.510  -3.547  1.00 31.00           N
ATOM   1594  CA  GLY A 632      24.308   7.152  -4.474  1.00 31.53           C
ATOM   1597  C   GLY A 632      23.842   6.238  -5.599  1.00 31.81           C
ATOM   1598  O   GLY A 632      24.651   5.877  -6.453  1.00 32.08           O
ATOM   1599  N   LYS A 633      22.566   5.842  -5.575  1.00 32.51           N
ATOM   1601  CA  LYS A 633      21.926   5.037  -6.631  1.00 33.28           C
ATOM   1603  CB  LYS A 633      21.631   5.921  -7.843  1.00 34.01           C
ATOM   1606  CG  LYS A 633      20.450   6.887  -7.619  1.00 34.40           C
ATOM   1609  CD  LYS A 633      20.242   7.895  -8.775  1.00 35.77           C
ATOM   1612  CE  LYS A 633      20.244   7.226 -10.165  1.00 36.91           C
ATOM   1615  NZ  LYS A 633      19.667   8.095 -11.259  1.00 34.73           N
ATOM   1619  C   LYS A 633      22.674   3.751  -7.031  1.00 33.99           C
ATOM   1620  O   LYS A 633      22.746   3.386  -8.200  1.00 34.69           O
ATOM   1621  N   ILE A 634      23.227   3.060  -6.039  1.00 34.33           N
ATOM   1623  CA  ILE A 634      23.870   1.772  -6.260  1.00 34.38           C
ATOM   1625  CB  ILE A 634      25.100   1.652  -5.329  1.00 34.92           C
ATOM   1627  CG1 ILE A 634      26.218   2.516  -5.884  1.00 35.10           C
ATOM   1630  CD1 ILE A 634      27.081   2.989  -4.854  1.00 37.95           C
ATOM   1634  CG2 ILE A 634      25.615   0.176  -5.155  1.00 35.62           C
ATOM   1638  C   ILE A 634      22.855   0.623  -6.091  1.00 34.47           C
ATOM   1639  O   ILE A 634      23.134  -0.519  -6.458  1.00 33.68           O
ATOM   1640  N   GLN A 635      21.671   0.943  -5.567  1.00 34.21           N
ATOM   1642  CA  GLN A 635      20.625  -0.049  -5.353  1.00 34.80           C
ATOM   1644  CB  GLN A 635      19.403   0.601  -4.706  1.00 35.03           C
ATOM   1647  CG  GLN A 635      18.209  -0.315  -4.540  1.00 36.49           C
ATOM   1650  CD  GLN A 635      17.084   0.307  -3.721  1.00 39.00           C
ATOM   1651  OE1 GLN A 635      16.900   1.526  -3.705  1.00 39.26           O
ATOM   1652  NE2 GLN A 635      16.318  -0.540  -3.055  1.00 42.25           N
ATOM   1655  C   GLN A 635      20.231  -0.754  -6.670  1.00 35.04           C
ATOM   1656  O   GLN A 635      20.076  -1.975  -6.685  1.00 33.84           O
ATOM   1657  N   ASN A 636      20.085   0.041  -7.741  1.00 35.20           N
ATOM   1659  CA  ASN A 636      19.770  -0.431  -9.090  1.00 36.02           C
ATOM   1661  CB  ASN A 636      19.655   0.738 -10.103  1.00 36.68           C
ATOM   1664  CG  ASN A 636      18.818   1.904  -9.595  1.00 39.99           C
ATOM   1665  OD1 ASN A 636      18.140   1.803  -8.565  1.00 46.78           O
ATOM   1666  ND2 ASN A 636      18.861   3.030 -10.321  1.00 42.53           N
ATOM   1669  C   ASN A 636      20.802  -1.389  -9.666  1.00 35.44           C
ATOM   1670  O   ASN A 636      20.492  -2.168 -10.552  1.00 35.08           O
ATOM   1671  N   LYS A 637      22.038  -1.296  -9.192  1.00 35.20           N
ATOM   1673  CA  LYS A 637      23.119  -2.130  -9.682  1.00 34.87           C
ATOM   1675  CB  LYS A 637      24.436  -1.348  -9.650  1.00 35.40           C
ATOM   1678  CG  LYS A 637      24.484  -0.150 -10.616  1.00 36.49           C
ATOM   1681  CD  LYS A 637      25.718   0.730 -10.327  1.00 38.37           C
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ATOM   1684  CE  LYS A 637      25.539   2.148 -10.877  1.00 38.75           C
ATOM   1687  NZ  LYS A 637      25.609   2.142 -12.360  1.00 38.23           N
ATOM   1691  C   LYS A 637      23.291  -3.435  -8.913  1.00 34.18           C
ATOM   1692  O   LYS A 637      24.107  -4.258  -9.304  1.00 33.84           O
ATOM   1693  N   LEU A 638      22.542  -3.628  -7.834  1.00 33.19           N
ATOM   1695  CA  LEU A 638      22.712  -4.805  -6.979  1.00 32.72           C
ATOM   1697  CB  LEU A 638      23.186  -4.369  -5.581  1.00 32.57           C
ATOM   1700  CG  LEU A 638      24.543  -3.658  -5.477  1.00 32.78           C
ATOM   1702  CD1 LEU A 638      24.915  -3.512  -4.038  1.00 33.08           C
ATOM   1706  CD2 LEU A 638      25.653  -4.387  -6.192  1.00 34.28           C
ATOM   1710  C   LEU A 638      21.423  -5.635  -6.858  1.00 32.44           C
ATOM   1711  O   LEU A 638      20.320  -5.133  -7.095  1.00 32.46           O
ATOM   1712  N   THR A 639      21.558  -6.904  -6.477  1.00 31.41           N
ATOM   1714  CA  THR A 639      20.383  -7.763  -6.278  1.00 31.03           C
ATOM   1716  CB  THR A 639      20.717  -9.246  -6.548  1.00 30.99           C
ATOM   1718  OG1 THR A 639      21.643  -9.717  -5.552  1.00 29.09           O
ATOM   1720  CG2 THR A 639      21.409  -9.440  -7.920  1.00 30.02           C
ATOM   1724  C   THR A 639      19.885  -7.644  -4.848  1.00 30.57           C
ATOM   1725  O   THR A 639      20.586  -7.138  -4.004  1.00 30.89           O
ATOM   1726  N   ASP A 640      18.691  -8.158  -4.585  1.00 30.85           N
ATOM   1728  CA  ASP A 640      18.121  -8.202  -3.245  1.00 31.53           C
ATOM   1730  CB  ASP A 640      16.742  -8.853  -3.244  1.00 32.21           C
ATOM   1733  CG  ASP A 640      15.663  -7.950  -3.781  1.00 36.04           C
ATOM   1734  OD1 ASP A 640      15.767  -6.715  -3.599  1.00 38.91           O
ATOM   1735  OD2 ASP A 640      14.668  -8.407  -4.387  1.00 41.38           O
ATOM   1736  C   ASP A 640      18.997  -8.962  -2.263  1.00 31.29           C
ATOM   1737  O   ASP A 640      19.163  -8.503  -1.146  1.00 31.11           O
ATOM   1738  N   LEU A 641      19.533 -10.115  -2.665  1.00 31.09           N
ATOM   1740  CA  LEU A 641      20.416 -10.897  -1.787  1.00 31.26           C
ATOM   1742  CB  LEU A 641      20.779 -12.249  -2.421  1.00 31.33           C
ATOM   1745  CG  LEU A 641      19.675 -13.291  -2.564  1.00 30.70           C
ATOM   1747  CD1 LEU A 641      20.271 -14.613  -3.042  1.00 30.42           C
ATOM   1751  CD2 LEU A 641      18.875 -13.482  -1.261  1.00 29.87           C
ATOM   1755  C   LEU A 641      21.685 -10.134  -1.449  1.00 30.81           C
ATOM   1756  O   LEU A 641      22.119 -10.146  -0.312  1.00 31.86           O
ATOM   1757  N   GLU A 642      22.259  -9.449  -2.431  1.00 30.40           N
ATOM   1759  CA  GLU A 642      23.483  -8.674  -2.225  1.00 30.27           C
ATOM   1761  CB  GLU A 642      24.032  -8.166  -3.558  1.00 30.21           C
ATOM   1764  CG  GLU A 642      24.558  -9.287  -4.455  1.00 30.03           C
ATOM   1767  CD  GLU A 642      24.788  -8.881  -5.910  1.00 32.15           C
ATOM   1768  OE1 GLU A 642      24.321  -7.805  -6.335  1.00 33.95           O
ATOM   1769  OE2 GLU A 642      25.416  -9.657  -6.666  1.00 32.81           O
ATOM   1770  C   GLU A 642      23.245  -7.528  -1.250  1.00 30.47           C
ATOM   1771  O   GLU A 642      24.048  -7.293  -0.342  1.00 30.71           O
ATOM   1772  N   ILE A 643      22.099  -6.873  -1.401  1.00 30.77           N
ATOM   1774  CA  ILE A 643      21.713  -5.760  -0.569  1.00 30.94           C
ATOM   1776  CB  ILE A 643      20.501  -5.040  -1.122  1.00 30.64           C
ATOM   1778  CG1 ILE A 643      20.882  -4.330  -2.424  1.00 31.48           C
ATOM   1781  CD1 ILE A 643      19.691  -3.841  -3.235  1.00 30.95           C
ATOM   1785  CG2 ILE A 643      19.980  -4.033  -0.117  1.00 31.85           C
ATOM   1789  C   ILE A 643      21.482  -6.207   0.866  1.00 31.22           C
ATOM   1790  O   ILE A 643      21.996  -5.568   1.776  1.00 30.90           O
ATOM   1791  N   LEU A 644      20.753  -7.302   1.048  1.00 30.89           N
ATOM   1793  CA  LEU A 644      20.533  -7.934   2.357  1.00 31.35           C
ATOM   1795  CB  LEU A 644      19.684  -9.204   2.158  1.00 31.85           C
ATOM   1798  CG  LEU A 644      19.344 -10.070   3.376  1.00 32.51           C
ATOM   1800  CD1 LEU A 644      18.561  -9.281   4.419  1.00 31.68           C
ATOM   1804  CD2 LEU A 644      18.557 -11.302   2.924  1.00 34.35           C
ATOM   1808  C   LEU A 644      21.837  -8.298   3.053  1.00 31.35           C
ATOM   1809  O   LEU A 644      22.039  -7.976   4.214  1.00 32.00           O
ATOM   1810  N   ALA A 645      22.746  -8.922   2.320  1.00 30.91           N
ATOM   1812  CA  ALA A 645      23.987  -9.374   2.897  1.00 31.28           C
ATOM   1814  CB  ALA A 645      24.735 -10.286   1.944  1.00 30.79           C
ATOM   1818  C   ALA A 645      24.864  -8.181   3.254  1.00 31.60           C
ATOM   1819  O   ALA A 645      25.548  -8.234   4.244  1.00 32.24           O
ATOM   1820  N   LEU A 646      24.854  -7.138   2.431  1.00 31.70           N
ATOM   1822  CA  LEU A 646      25.726  -5.985   2.633  1.00 32.69           C
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ATOM	1824	CB	LEU	A	646	25.701	-5.004	1.465	1.00	33.10	C
ATOM	1827	CG	LEU	A	646	26.465	-5.375	0.202	1.00	36.19	C
ATOM	1829	CD1	LEU	A	646	25.967	-4.482	-0.948	1.00	37.84	C
ATOM	1833	CD2	LEU	A	646	27.950	-5.267	0.411	1.00	37.50	C
ATOM	1837	C	LEU	A	646	25.291	-5.242	3.861	1.00	32.76	C
ATOM	1838	O	LEU	A	646	26.122	-4.810	4.627	1.00	32.57	O
ATOM	1839	N	LEU	A	647	23.985	-5.094	4.035	1.00	33.13	N
ATOM	1841	CA	LEU	A	647	23.457	-4.468	5.237	1.00	33.42	C
ATOM	1843	CB	LEU	A	647	21.947	-4.256	5.101	1.00	33.13	C
ATOM	1846	CG	LEU	A	647	21.357	-3.326	6.155	1.00	34.76	C
ATOM	1848	CD1	LEU	A	647	22.086	-1.969	6.182	1.00	34.70	C
ATOM	1852	CD2	LEU	A	647	19.850	-3.117	5.959	1.00	35.72	C
ATOM	1856	C	LEU	A	647	23.815	-5.284	6.509	1.00	33.49	C
ATOM	1857	O	LEU	A	647	24.288	-4.735	7.519	1.00	33.49	O
ATOM	1858	N	ILE	A	648	23.657	-6.596	6.450	1.00	33.09	N
ATOM	1860	CA	ILE	A	648	23.917	-7.413	7.618	1.00	32.95	C
ATOM	1862	CB	ILE	A	648	23.412	-8.832	7.423	1.00	32.70	C
ATOM	1864	CG1	ILE	A	648	21.876	-8.857	7.471	1.00	32.59	C
ATOM	1867	CD1	ILE	A	648	21.271	-10.103	6.822	1.00	33.18	C
ATOM	1871	CG2	ILE	A	648	24.015	-9.759	8.477	1.00	33.03	C
ATOM	1875	C	ILE	A	648	25.405	-7.411	7.965	1.00	33.03	C
ATOM	1876	O	ILE	A	648	25.755	-7.312	9.138	1.00	32.99	O
ATOM	1877	N	ALA	A	649	26.252	-7.529	6.943	1.00	32.46	N
ATOM	1879	CA	ALA	A	649	27.694	-7.441	7.091	1.00	32.19	C
ATOM	1881	CB	ALA	A	649	28.376	-7.690	5.747	1.00	31.91	C
ATOM	1885	C	ALA	A	649	28.166	-6.112	7.683	1.00	31.99	C
ATOM	1886	O	ALA	A	649	28.959	-6.097	8.603	1.00	31.02	O
ATOM	1887	N	ALA	A	650	27.667	-4.998	7.162	1.00	32.64	N
ATOM	1889	CA	ALA	A	650	28.030	-3.672	7.686	1.00	32.16	C
ATOM	1891	CB	ALA	A	650	27.265	-2.592	6.957	1.00	32.87	C
ATOM	1895	C	ALA	A	650	27.754	-3.599	9.167	1.00	31.97	C
ATOM	1896	O	ALA	A	650	28.619	-3.171	9.962	1.00	31.46	O
ATOM	1897	N	LEU	A	651	26.582	-4.081	9.556	1.00	31.43	N
ATOM	1899	CA	LEU	A	651	26.159	-4.076	10.963	1.00	31.21	C
ATOM	1901	CB	LEU	A	651	24.673	-4.404	11.083	1.00	31.32	C
ATOM	1904	CG	LEU	A	651	23.742	-3.326	10.542	1.00	32.12	C
ATOM	1906	CD1	LEU	A	651	22.344	-3.843	10.355	1.00	32.83	C
ATOM	1910	CD2	LEU	A	651	23.743	-2.125	11.438	1.00	32.23	C
ATOM	1914	C	LEU	A	651	26.957	-5.047	11.850	1.00	31.12	C
ATOM	1915	O	LEU	A	651	27.210	-4.751	13.011	1.00	29.74	O
ATOM	1916	N	SER	A	652	27.396	-6.167	11.277	1.00	31.07	N
ATOM	1918	CA	SER	A	652	28.029	-7.252	12.038	1.00	31.40	C
ATOM	1920	CB	SER	A	652	27.488	-8.590	11.545	1.00	31.66	C
ATOM	1923	OG	SER	A	652	26.075	-8.596	11.563	1.00	33.22	O
ATOM	1925	C	SER	A	652	29.551	-7.369	11.982	1.00	30.56	C
ATOM	1926	O	SER	A	652	30.117	-8.177	12.697	1.00	28.98	O
ATOM	1927	N	HIS	A	653	30.212	-6.586	11.144	1.00	30.93	N
ATOM	1929	CA	HIS	A	653	31.583	-6.901	10.760	1.00	31.59	C
ATOM	1931	CB	HIS	A	653	32.037	-6.037	9.578	1.00	31.74	C
ATOM	1934	CG	HIS	A	653	32.262	-4.609	9.935	1.00	32.86	C
ATOM	1935	ND1	HIS	A	653	31.236	-3.705	10.043	1.00	34.83	N
ATOM	1937	CE1	HIS	A	653	31.724	-2.526	10.380	1.00	33.06	C
ATOM	1939	NE2	HIS	A	653	33.035	-2.632	10.474	1.00	34.35	N
ATOM	1941	CD2	HIS	A	653	33.397	-3.922	10.205	1.00	34.04	C
ATOM	1943	C	HIS	A	653	32.610	-6.765	11.900	1.00	31.77	C
ATOM	1944	O	HIS	A	653	33.694	-7.343	11.786	1.00	31.53	O
ATOM	1945	N	ASP	A	654	32.287	-6.016	12.967	1.00	31.02	N
ATOM	1947	CA	ASP	A	654	33.203	-5.869	14.110	1.00	32.06	C
ATOM	1949	CB	ASP	A	654	33.267	-4.406	14.541	1.00	31.95	C
ATOM	1952	CG	ASP	A	654	34.342	-3.613	13.798	1.00	34.24	C
ATOM	1953	OD1	ASP	A	654	35.199	-4.243	13.103	1.00	32.30	O
ATOM	1954	OD2	ASP	A	654	34.419	-2.361	13.876	1.00	34.59	O
ATOM	1955	C	ASP	A	654	32.867	-6.772	15.296	1.00	33.06	C
ATOM	1956	O	ASP	A	654	33.422	-6.624	16.379	1.00	31.77	O
ATOM	1957	N	LEU	A	655	31.933	-7.703	15.108	1.00	34.41	N
ATOM	1959	CA	LEU	A	655	31.696	-8.763	16.103	1.00	36.09	C
ATOM	1961	CB	LEU	A	655	30.302	-9.352	15.905	1.00	35.24	C

ATOM	1964	CG	LEU	A	655	29.159	-8.361	16.026	1.00	35.88	C
ATOM	1966	CD1	LEU	A	655	27.882	-8.973	15.468	1.00	37.30	C
ATOM	1970	CD2	LEU	A	655	29.000	-7.937	17.479	1.00	36.08	C
ATOM	1974	C	LEU	A	655	32.749	-9.857	15.921	1.00	37.80	C
ATOM	1975	O	LEU	A	655	32.686	-10.587	14.946	1.00	38.36	O
ATOM	1976	N	ASP	A	656	33.700	-10.006	16.838	1.00	40.55	N
ATOM	1978	CA	ASP	A	656	34.920	-10.783	16.530	1.00	42.89	C
ATOM	1980	CB	ASP	A	656	36.142	-9.844	16.644	1.00	44.08	C
ATOM	1983	CG	ASP	A	656	37.298	-10.225	15.710	1.00	48.10	C
ATOM	1984	OD1	ASP	A	656	37.051	-10.684	14.565	1.00	52.44	O
ATOM	1985	OD2	ASP	A	656	38.508	-10.062	16.043	1.00	54.53	O
ATOM	1986	C	ASP	A	656	35.176	-12.045	17.381	1.00	43.63	C
ATOM	1987	O	ASP	A	656	36.266	-12.637	17.299	1.00	43.86	O
ATOM	1988	N	HIS	A	657	34.202	-12.481	18.176	1.00	44.15	N
ATOM	1990	CA	HIS	A	657	34.493	-13.496	19.209	1.00	44.68	C
ATOM	1992	CB	HIS	A	657	33.333	-13.615	20.208	1.00	44.74	C
ATOM	1995	CG	HIS	A	657	33.742	-14.123	21.562	1.00	46.30	C
ATOM	1996	ND1	HIS	A	657	32.944	-14.961	22.319	1.00	47.41	N
ATOM	1998	CE1	HIS	A	657	33.557	-15.248	23.455	1.00	47.05	C
ATOM	2000	NE2	HIS	A	657	34.726	-14.630	23.465	1.00	47.08	N
ATOM	2002	CD2	HIS	A	657	34.866	-13.920	22.294	1.00	47.25	C
ATOM	2004	C	HIS	A	657	34.902	-14.885	18.649	1.00	44.72	C
ATOM	2005	O	HIS	A	657	34.141	-15.568	17.959	1.00	44.45	O
ATOM	2006	N	LEU	A	672	45.942	-7.901	25.006	1.00	40.22	N
ATOM	2008	CA	LEU	A	672	44.765	-7.106	25.373	1.00	39.72	C
ATOM	2010	CB	LEU	A	672	44.699	-6.859	26.913	1.00	39.47	C
ATOM	2013	CG	LEU	A	672	43.415	-7.305	27.648	1.00	41.50	C
ATOM	2015	CD1	LEU	A	672	43.452	-6.944	29.155	1.00	42.61	C
ATOM	2019	CD2	LEU	A	672	42.112	-6.783	27.000	1.00	42.23	C
ATOM	2023	C	LEU	A	672	44.744	-5.790	24.557	1.00	38.16	C
ATOM	2024	O	LEU	A	672	43.725	-5.460	23.975	1.00	39.19	O
ATOM	2025	N	ALA	A	673	45.862	-5.067	24.492	1.00	37.22	N
ATOM	2027	CA	ALA	A	673	45.987	-3.837	23.650	1.00	35.47	C
ATOM	2029	CB	ALA	A	673	47.445	-3.430	23.512	1.00	35.51	C
ATOM	2033	C	ALA	A	673	45.363	-3.964	22.279	1.00	34.63	C
ATOM	2034	O	ALA	A	673	45.567	-4.963	21.609	1.00	34.37	O
ATOM	2035	N	GLN	A	674	44.559	-2.973	21.877	1.00	33.43	N
ATOM	2037	CA	GLN	A	674	43.895	-2.994	20.595	1.00	33.60	C
ATOM	2039	CB	GLN	A	674	42.441	-2.497	20.707	1.00	34.24	C
ATOM	2042	CG	GLN	A	674	41.513	-3.452	21.486	1.00	37.75	C
ATOM	2045	CD	GLN	A	674	40.544	-4.242	20.641	1.00	38.80	C
ATOM	2046	OE1	GLN	A	674	40.254	-3.910	19.474	1.00	44.44	O
ATOM	2047	NE2	GLN	A	674	39.998	-5.288	21.241	1.00	43.56	N
ATOM	2050	C	GLN	A	674	44.636	-2.149	19.546	1.00	32.40	C
ATOM	2051	O	GLN	A	674	45.186	-1.073	19.851	1.00	31.00	O
ATOM	2052	N	LEU	A	675	44.624	-2.667	18.328	1.00	30.90	N
ATOM	2054	CA	LEU	A	675	45.211	-2.030	17.161	1.00	31.32	C
ATOM	2056	CB	LEU	A	675	45.047	-2.949	15.927	1.00	30.92	C
ATOM	2059	CG	LEU	A	675	45.868	-4.231	15.922	1.00	31.96	C
ATOM	2061	CD1	LEU	A	675	45.312	-5.271	14.935	1.00	32.94	C
ATOM	2065	CD2	LEU	A	675	47.374	-3.924	15.643	1.00	31.58	C
ATOM	2069	C	LEU	A	675	44.462	-0.744	16.887	1.00	31.01	C
ATOM	2070	O	LEU	A	675	43.268	-0.643	17.182	1.00	31.24	O
ATOM	2071	N	TYR	A	676	45.154	0.234	16.326	1.00	30.42	N
ATOM	2073	CA	TYR	A	676	44.500	1.431	15.814	1.00	30.40	C
ATOM	2075	CB	TYR	A	676	45.470	2.333	15.026	1.00	30.49	C
ATOM	2078	CG	TYR	A	676	45.057	3.757	15.120	1.00	29.31	C
ATOM	2079	CD1	TYR	A	676	45.421	4.496	16.204	1.00	29.50	C
ATOM	2081	CE1	TYR	A	676	45.044	5.813	16.339	1.00	31.10	C
ATOM	2083	CZ	TYR	A	676	44.234	6.398	15.399	1.00	28.90	C
ATOM	2084	OH	TYR	A	676	43.874	7.712	15.604	1.00	33.89	O
ATOM	2086	CE2	TYR	A	676	43.817	5.686	14.301	1.00	30.81	C
ATOM	2088	CD2	TYR	A	676	44.240	4.351	14.151	1.00	30.43	C
ATOM	2090	C	TYR	A	676	43.347	1.066	14.920	1.00	31.11	C
ATOM	2091	O	TYR	A	676	43.381	0.042	14.239	1.00	30.91	O
ATOM	2092	N	CYS	A	677	42.325	1.903	14.918	1.00	32.33	N
ATOM	2094	CA	CYS	A	677	41.246	1.853	13.902	1.00	34.86	C

ATOM	2096	CB	CYS	A	677	40.555	3.228	13.918	1.00	35.17	C
ATOM	2099	SG	CYS	A	677	38.936	3.136	13.268	1.00	45.40	S
ATOM	2100	C	CYS	A	677	41.718	1.627	12.430	1.00	33.82	C
ATOM	2101	O	CYS	A	677	42.504	2.406	11.920	1.00	33.14	O
ATOM	2102	N	HIS	A	678	41.188	0.596	11.765	1.00	34.07	N
ATOM	2104	CA	HIS	A	678	41.471	0.227	10.363	1.00	33.24	C
ATOM	2106	CB	HIS	A	678	41.112	1.358	9.401	1.00	34.61	C
ATOM	2109	CG	HIS	A	678	39.763	1.949	9.632	1.00	35.54	C
ATOM	2110	ND1	HIS	A	678	38.602	1.205	9.588	1.00	37.52	N
ATOM	2112	CE1	HIS	A	678	37.569	1.999	9.805	1.00	34.44	C
ATOM	2114	NE2	HIS	A	678	38.017	3.225	9.985	1.00	35.87	N
ATOM	2116	CD2	HIS	A	678	39.386	3.220	9.885	1.00	35.92	C
ATOM	2118	C	HIS	A	678	42.899	-0.232	10.041	1.00	33.03	C
ATOM	2119	O	HIS	A	678	43.323	-0.198	8.892	1.00	33.36	O
ATOM	2120	N	SER	A	679	43.630	-0.677	11.049	1.00	31.57	N
ATOM	2122	CA	SER	A	679	44.947	-1.228	10.870	1.00	30.05	C
ATOM	2124	CB	SER	A	679	45.432	-1.789	12.208	1.00	30.03	C
ATOM	2127	OG	SER	A	679	46.508	-2.685	12.022	1.00	27.55	O
ATOM	2129	C	SER	A	679	44.933	-2.374	9.854	1.00	30.06	C
ATOM	2130	O	SER	A	679	44.007	-3.161	9.826	1.00	28.59	O
ATOM	2131	N	ILE	A	680	46.001	-2.458	9.064	1.00	29.54	N
ATOM	2133	CA	ILE	A	680	46.276	-3.552	8.133	1.00	30.65	C
ATOM	2135	CB	ILE	A	680	47.564	-3.218	7.299	1.00	31.62	C
ATOM	2137	CG1	ILE	A	680	47.396	-1.920	6.501	1.00	35.56	C
ATOM	2140	CD1	ILE	A	680	48.607	-1.711	5.515	1.00	38.92	C
ATOM	2144	CG2	ILE	A	680	47.878	-4.299	6.251	1.00	34.26	C
ATOM	2148	C	ILE	A	680	46.435	-4.895	8.846	1.00	30.33	C
ATOM	2149	O	ILE	A	680	46.355	-5.951	8.221	1.00	30.79	O
ATOM	2150	N	MET	A	681	46.642	-4.857	10.158	1.00	29.68	N
ATOM	2152	CA	MET	A	681	46.814	-6.057	10.931	1.00	30.17	C
ATOM	2154	CB	MET	A	681	47.828	-5.794	12.070	1.00	29.84	C
ATOM	2157	CG	MET	A	681	49.212	-5.461	11.539	1.00	31.17	C
ATOM	2160	SD	MET	A	681	49.737	-6.803	10.421	1.00	33.29	S
ATOM	2161	CE	MET	A	681	51.480	-6.883	10.639	1.00	34.00	C
ATOM	2165	C	MET	A	681	45.498	-6.637	11.473	1.00	30.11	C
ATOM	2166	O	MET	A	681	45.526	-7.716	11.991	1.00	29.99	O
ATOM	2167	N	GLU	A	682	44.371	-5.941	11.318	1.00	30.83	N
ATOM	2169	CA	GLU	A	682	43.083	-6.433	11.782	1.00	32.14	C
ATOM	2171	CB	GLU	A	682	42.002	-5.357	11.801	1.00	32.53	C
ATOM	2174	CG	GLU	A	682	42.241	-4.109	12.598	1.00	35.57	C
ATOM	2177	CD	GLU	A	682	41.150	-3.074	12.378	1.00	38.08	C
ATOM	2178	OE1	GLU	A	682	40.351	-3.244	11.428	1.00	38.05	O
ATOM	2179	OE2	GLU	A	682	41.092	-2.089	13.152	1.00	38.67	O
ATOM	2180	C	GLU	A	682	42.562	-7.526	10.868	1.00	32.35	C
ATOM	2181	O	GLU	A	682	42.722	-7.455	9.649	1.00	32.87	O
ATOM	2182	N	HIS	A	683	41.912	-8.515	11.468	1.00	32.50	N
ATOM	2184	CA	HIS	A	683	41.286	-9.593	10.721	1.00	33.32	C
ATOM	2186	CB	HIS	A	683	42.201	-10.824	10.637	1.00	32.66	C
ATOM	2189	CG	HIS	A	683	43.316	-10.596	9.677	1.00	34.89	C
ATOM	2190	ND1	HIS	A	683	43.082	-10.378	8.328	1.00	34.82	N
ATOM	2192	CE1	HIS	A	683	44.226	-10.085	7.733	1.00	34.74	C
ATOM	2194	NE2	HIS	A	683	45.183	-10.065	8.649	1.00	36.27	N
ATOM	2196	CD2	HIS	A	683	44.631	-10.343	9.880	1.00	34.25	C
ATOM	2198	C	HIS	A	683	39.924	-9.873	11.311	1.00	33.32	C
ATOM	2199	O	HIS	A	683	39.811	-10.225	12.450	1.00	33.83	O
ATOM	2200	N	HIS	A	684	38.899	-9.696	10.503	1.00	33.32	N
ATOM	2202	CA	HIS	A	684	37.542	-9.800	10.968	1.00	33.77	C
ATOM	2204	CB	HIS	A	684	36.660	-8.837	10.181	1.00	33.24	C
ATOM	2207	CG	HIS	A	684	37.150	-7.446	10.227	1.00	35.04	C
ATOM	2208	ND1	HIS	A	684	36.610	-6.499	11.072	1.00	37.86	N
ATOM	2210	CE1	HIS	A	684	37.266	-5.365	10.921	1.00	34.96	C
ATOM	2212	NE2	HIS	A	684	38.233	-5.553	10.043	1.00	33.74	N
ATOM	2214	CD2	HIS	A	684	38.188	-6.850	9.604	1.00	32.56	C
ATOM	2216	C	HIS	A	684	37.073	-11.221	10.762	1.00	33.63	C
ATOM	2217	O	HIS	A	684	37.579	-11.936	9.898	1.00	34.39	O
ATOM	2218	N	HIS	A	685	36.103	-11.618	11.563	1.00	33.78	N
ATOM	2220	CA	HIS	A	685	35.509	-12.946	11.462	1.00	34.15	C

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ATOM   2222  CB  HIS A 685      35.955 -13.834  12.635  1.00 34.73           C
ATOM   2225  CG  HIS A 685      37.444 -13.941  12.772  1.00 37.59           C
ATOM   2226  ND1 HIS A 685      38.212 -14.721  11.933  1.00 42.39           N
ATOM   2228  CE1 HIS A 685      39.486 -14.589  12.255  1.00 41.15           C
ATOM   2230  NE2 HIS A 685      39.574 -13.749  13.268  1.00 40.09           N
ATOM   2232  CD2 HIS A 685      38.314 -13.318  13.603  1.00 39.95           C
ATOM   2234  C   HIS A 685      33.974 -12.866  11.360  1.00 32.94           C
ATOM   2235  O   HIS A 685      33.335 -11.946  11.820  1.00 32.00           O
ATOM   2236  N   PHE A 686      33.412 -13.870  10.741  1.00 33.17           N
ATOM   2238  CA  PHE A 686      32.008 -13.945  10.489  1.00 33.88           C
ATOM   2240  CB  PHE A 686      31.803 -14.491   9.088  1.00 35.01           C
ATOM   2243  CG  PHE A 686      32.525 -15.772   8.780  1.00 38.39           C
ATOM   2244  CD1 PHE A 686      32.167 -16.981   9.392  1.00 41.03           C
ATOM   2246  CE1 PHE A 686      32.803 -18.183   9.051  1.00 40.91           C
ATOM   2248  CZ  PHE A 686      33.804 -18.187   8.078  1.00 42.36           C
ATOM   2250  CE2 PHE A 686      34.157 -17.002   7.438  1.00 41.72           C
ATOM   2252  CD2 PHE A 686      33.515 -15.795   7.787  1.00 42.86           C
ATOM   2254  C   PHE A 686      31.292 -14.815  11.511  1.00 33.38           C
ATOM   2255  O   PHE A 686      30.065 -14.879  11.546  1.00 33.04           O
ATOM   2259  CG  PHE B 686      29.676 -15.568   8.280  1.00 50.77           C
ATOM   2260  CD1 PHE B 686      28.658 -14.648   8.564  1.00 51.19           C
ATOM   2262  CE1 PHE B 686      27.557 -14.841   7.742  1.00 51.49           C
ATOM   2264  CZ  PHE B 686      27.349 -16.162   7.300  1.00 51.14           C
ATOM   2266  CE2 PHE B 686      27.994 -17.198   8.051  1.00 51.13           C
ATOM   2268  CD2 PHE B 686      29.294 -16.908   8.397  1.00 50.89           C
ATOM   2272  N   ASP A 687      32.092 -15.582  12.599  1.00 31.50           N
ATOM   2274  CA  ASP A 687      31.473 -16.638  13.419  1.00 32.97           C
ATOM   2276  CB  ASP A 687      32.527 -17.298  14.298  1.00 33.51           C
ATOM   2279  CG  ASP A 687      33.782 -17.750  13.505  1.00 37.66           C
ATOM   2280  OD1 ASP A 687      34.365 -16.976  12.688  1.00 41.70           O
ATOM   2281  OD2 ASP A 687      34.275 -18.883  13.661  1.00 43.47           O
ATOM   2282  C   ASP A 687      30.352 -16.037  14.265  1.00 32.03           C
ATOM   2283  O   ASP A 687      29.286 -16.621  14.357  1.00 32.15           O
ATOM   2286  N   GLN A 688      30.565 -14.851  14.836  1.00 31.16           N
ATOM   2288  CA  GLN A 688      29.589 -14.264  15.750  1.00 31.25           C
ATOM   2290  CB  GLN A 688      30.225 -13.154  16.582  1.00 31.77           C
ATOM   2293  CG  GLN A 688      29.374 -12.759  17.799  1.00 34.88           C
ATOM   2296  CD  GLN A 688      30.119 -11.885  18.796  1.00 38.18           C
ATOM   2297  OE1 GLN A 688      31.181 -11.329  18.485  1.00 40.54           O
ATOM   2298  NE2 GLN A 688      29.559 -11.755  19.992  1.00 39.53           N
ATOM   2301  C   GLN A 688      28.353 -13.730  15.017  1.00 31.05           C
ATOM   2302  O   GLN A 688      27.220 -13.846  15.518  1.00 30.38           O
ATOM   2303  N   CYS A 689      28.555 -13.163  13.827  1.00 30.12           N
ATOM   2305  CA  CYS A 689      27.435 -12.763  12.967  1.00 30.44           C
ATOM   2307  CB  CYS A 689      27.949 -12.200  11.644  1.00 30.42           C
ATOM   2310  SG  CYS A 689      26.668 -11.902  10.426  1.00 30.66           S
ATOM   2311  C   CYS A 689      26.467 -13.923  12.677  1.00 30.97           C
ATOM   2312  O   CYS A 689      25.250 -13.779  12.825  1.00 31.28           O
ATOM   2313  N   LEU A 690      27.015 -15.064  12.276  1.00 31.79           N
ATOM   2315  CA  LEU A 690      26.236 -16.264  11.933  1.00 32.94           C
ATOM   2317  CB  LEU A 690      27.184 -17.331  11.368  1.00 33.46           C
ATOM   2320  CG  LEU A 690      26.757 -18.703  10.824  1.00 37.99           C
ATOM   2322  CD1 LEU A 690      26.703 -19.815  11.914  1.00 40.57           C
ATOM   2326  CD2 LEU A 690      25.420 -18.617  10.058  1.00 40.70           C
ATOM   2330  C   LEU A 690      25.538 -16.818  13.171  1.00 32.70           C
ATOM   2331  O   LEU A 690      24.375 -17.172  13.118  1.00 32.47           O
ATOM   2332  N   MET A 691      26.265 -16.907  14.284  1.00 33.18           N
ATOM   2334  CA  MET A 691      25.691 -17.403  15.534  1.00 34.16           C
ATOM   2336  CB  MET A 691      26.723 -17.390  16.651  1.00 35.01           C
ATOM   2339  CG  MET A 691      26.091 -17.561  18.050  1.00 40.17           C
ATOM   2342  SD  MET A 691      27.311 -17.341  19.371  1.00 50.88           S
ATOM   2343  CE  MET A 691      27.597 -15.483  19.419  1.00 47.95           C
ATOM   2347  C   MET A 691      24.449 -16.590  15.950  1.00 33.27           C
ATOM   2348  O   MET A 691      23.448 -17.160  16.358  1.00 32.30           O
ATOM   2349  N   ILE A 692      24.506 -15.266  15.816  1.00 32.39           N
ATOM   2351  CA  ILE A 692      23.360 -14.430  16.191  1.00 32.77           C
ATOM   2353  CB  ILE A 692      23.768 -12.937  16.271  1.00 32.53           C
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ATOM   2355  CG1 ILE A 692      24.784 -12.735  17.400  1.00 32.90           C
ATOM   2358  CD1 ILE A 692      25.376 -11.322  17.429  1.00 34.91           C
ATOM   2362  CG2 ILE A 692      22.547 -12.060  16.506  1.00 33.18           C
ATOM   2366  C   ILE A 692      22.173 -14.636  15.235  1.00 32.75           C
ATOM   2367  O   ILE A 692      21.019 -14.721  15.668  1.00 32.34           O
ATOM   2368  N   LEU A 693      22.476 -14.732  13.942  1.00 33.49           N
ATOM   2370  CA  LEU A 693      21.493 -14.980  12.882  1.00 33.88           C
ATOM   2372  CB  LEU A 693      22.191 -15.052  11.524  1.00 33.93           C
ATOM   2375  CG  LEU A 693      22.548 -13.756  10.805  1.00 35.20           C
ATOM   2377  CD1 LEU A 693      23.319 -14.084   9.540  1.00 35.75           C
ATOM   2381  CD2 LEU A 693      21.313 -12.992  10.457  1.00 37.26           C
ATOM   2385  C   LEU A 693      20.739 -16.291  13.056  1.00 34.18           C
ATOM   2386  O   LEU A 693      19.615 -16.427  12.571  1.00 34.35           O
ATOM   2387  N   ASN A 694      21.379 -17.266  13.690  1.00 34.61           N
ATOM   2389  CA  ASN A 694      20.787 -18.578  13.893  1.00 35.26           C
ATOM   2391  CB  ASN A 694      21.823 -19.662  13.585  1.00 36.11           C
ATOM   2394  CG  ASN A 694      22.160 -19.729  12.109  1.00 39.88           C
ATOM   2395  OD1 ASN A 694      21.322 -19.374  11.254  1.00 44.53           O
ATOM   2396  ND2 ASN A 694      23.390 -20.182  11.789  1.00 41.14           N
ATOM   2399  C   ASN A 694      20.231 -18.782  15.305  1.00 34.38           C
ATOM   2400  O   ASN A 694      19.838 -19.874  15.660  1.00 33.37           O
ATOM   2401  N   SER A 695      20.202 -17.715  16.089  1.00 33.65           N
ATOM   2403  CA  SER A 695      19.858 -17.780  17.499  1.00 33.45           C
ATOM   2405  CB  SER A 695      20.478 -16.562  18.191  1.00 33.53           C
ATOM   2408  OG  SER A 695      20.716 -16.834  19.539  1.00 36.56           O
ATOM   2410  C   SER A 695      18.347 -17.737  17.615  1.00 32.46           C
ATOM   2411  O   SER A 695      17.735 -16.981  16.874  1.00 31.34           O
ATOM   2412  N   PRO A 696      17.726 -18.528  18.506  1.00 32.72           N
ATOM   2413  CA  PRO A 696      16.253 -18.548  18.596  1.00 32.48           C
ATOM   2415  CB  PRO A 696      15.977 -19.410  19.837  1.00 32.89           C
ATOM   2418  CG  PRO A 696      17.170 -20.303  19.949  1.00 33.37           C
ATOM   2421  CD  PRO A 696      18.342 -19.465  19.468  1.00 32.62           C
ATOM   2424  C   PRO A 696      15.662 -17.153  18.773  1.00 32.13           C
ATOM   2425  O   PRO A 696      16.197 -16.391  19.560  1.00 32.27           O
ATOM   2426  N   GLY A 697      14.611 -16.825  18.023  1.00 31.88           N
ATOM   2428  CA  GLY A 697      13.997 -15.503  18.056  1.00 31.92           C
ATOM   2431  C   GLY A 697      14.697 -14.389  17.276  1.00 31.76           C
ATOM   2432  O   GLY A 697      14.146 -13.287  17.133  1.00 31.96           O
ATOM   2433  N   ASN A 698      15.890 -14.665  16.754  1.00 31.26           N
ATOM   2435  CA  ASN A 698      16.699 -13.663  16.060  1.00 31.31           C
ATOM   2437  CB  ASN A 698      18.093 -13.574  16.697  1.00 31.05           C
ATOM   2440  CG  ASN A 698      18.073 -13.015  18.099  1.00 30.32           C
ATOM   2441  OD1 ASN A 698      18.380 -11.869  18.298  1.00 30.29           O
ATOM   2442  ND2 ASN A 698      17.731 -13.836  19.078  1.00 32.05           N
ATOM   2445  C   ASN A 698      16.877 -13.999  14.580  1.00 31.57           C
ATOM   2446  O   ASN A 698      17.687 -13.374  13.900  1.00 31.67           O
ATOM   2447  N   GLN A 699      16.134 -14.990  14.084  1.00 31.49           N
ATOM   2449  CA  GLN A 699      16.391 -15.548  12.763  1.00 31.46           C
ATOM   2451  CB  GLN A 699      15.949 -17.022  12.691  1.00 32.07           C
ATOM   2454  CG  GLN A 699      16.781 -17.939  13.585  1.00 33.35           C
ATOM   2457  CD  GLN A 699      16.171 -19.315  13.780  1.00 36.39           C
ATOM   2458  OE1 GLN A 699      16.710 -20.309  13.298  1.00 37.88           O
ATOM   2459  NE2 GLN A 699      15.057 -19.378  14.500  1.00 38.71           N
ATOM   2462  C   GLN A 699      15.747 -14.709  11.674  1.00 31.10           C
ATOM   2463  O   GLN A 699      14.718 -15.079  11.104  1.00 30.82           O
ATOM   2464  N   ILE A 700      16.387 -13.592  11.346  1.00 30.47           N
ATOM   2466  CA  ILE A 700      15.834 -12.693  10.338  1.00 30.47           C
ATOM   2468  CB  ILE A 700      16.514 -11.300  10.394  1.00 30.43           C
ATOM   2470  CG1 ILE A 700      18.009 -11.385  10.130  1.00 32.01           C
ATOM   2473  CD1 ILE A 700      18.675 -10.020   9.846  1.00 32.43           C
ATOM   2477  CG2 ILE A 700      16.250 -10.653  11.752  1.00 29.96           C
ATOM   2481  C   ILE A 700      15.870 -13.260   8.908  1.00 30.26           C
ATOM   2482  O   ILE A 700      15.255 -12.701   8.037  1.00 29.34           O
ATOM   2483  N   LEU A 701      16.606 -14.346   8.679  1.00 30.75           N
ATOM   2485  CA  LEU A 701      16.675 -14.984   7.362  1.00 31.28           C
ATOM   2487  CB  LEU A 701      18.131 -15.364   7.008  1.00 30.89           C
ATOM   2490  CG  LEU A 701      19.172 -14.244   7.069  1.00 32.55           C
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ATOM	2492	CD1	LEU	A	701	20.528	-14.746	6.632	1.00	33.04	C
ATOM	2496	CD2	LEU	A	701	18.737	-13.067	6.230	1.00	32.79	C
ATOM	2500	C	LEU	A	701	15.795	-16.219	7.268	1.00	31.50	C
ATOM	2501	O	LEU	A	701	15.999	-17.024	6.371	1.00	31.76	O
ATOM	2502	N	SER	A	702	14.805	-16.361	8.157	1.00	31.90	N
ATOM	2504	CA	SER	A	702	13.963	-17.574	8.201	1.00	31.30	C
ATOM	2506	CB	SER	A	702	13.101	-17.613	9.480	1.00	31.65	C
ATOM	2509	OG	SER	A	702	12.202	-16.514	9.552	1.00	32.37	O
ATOM	2511	C	SER	A	702	13.071	-17.704	6.966	1.00	30.98	C
ATOM	2512	O	SER	A	702	12.822	-18.811	6.494	1.00	30.26	O
ATOM	2513	N	GLY	A	703	12.652	-16.567	6.423	1.00	30.27	N
ATOM	2515	CA	GLY	A	703	11.878	-16.506	5.196	1.00	30.37	C
ATOM	2518	C	GLY	A	703	12.606	-16.916	3.907	1.00	30.71	C
ATOM	2519	O	GLY	A	703	11.953	-17.174	2.893	1.00	30.10	O
ATOM	2520	N	LEU	A	704	13.946	-16.971	3.936	1.00	30.77	N
ATOM	2522	CA	LEU	A	704	14.726	-17.371	2.765	1.00	30.56	C
ATOM	2524	CB	LEU	A	704	16.210	-16.996	2.909	1.00	30.10	C
ATOM	2527	CG	LEU	A	704	16.723	-15.587	3.249	1.00	34.32	C
ATOM	2529	CD1	LEU	A	704	18.193	-15.367	2.788	1.00	33.13	C
ATOM	2533	CD2	LEU	A	704	15.867	-14.494	2.720	1.00	37.04	C
ATOM	2537	C	LEU	A	704	14.635	-18.884	2.523	1.00	29.50	C
ATOM	2538	O	LEU	A	704	14.684	-19.668	3.452	1.00	29.10	O
ATOM	2539	N	SER	A	705	14.531	-19.281	1.261	1.00	28.82	N
ATOM	2541	CA	SER	A	705	14.737	-20.665	0.851	1.00	28.42	C
ATOM	2543	CB	SER	A	705	14.463	-20.819	-0.649	1.00	28.44	C
ATOM	2546	OG	SER	A	705	15.424	-20.071	-1.386	1.00	28.13	O
ATOM	2548	C	SER	A	705	16.175	-21.096	1.156	1.00	28.17	C
ATOM	2549	O	SER	A	705	17.065	-20.251	1.338	1.00	27.96	O
ATOM	2550	N	ILE	A	706	16.418	-22.400	1.213	1.00	28.12	N
ATOM	2552	CA	ILE	A	706	17.751	-22.857	1.578	1.00	28.61	C
ATOM	2554	CB	ILE	A	706	17.826	-24.376	1.797	1.00	29.31	C
ATOM	2556	CG1	ILE	A	706	17.437	-25.161	0.549	1.00	29.59	C
ATOM	2559	CD1	ILE	A	706	17.422	-26.641	0.794	1.00	33.51	C
ATOM	2563	CG2	ILE	A	706	16.906	-24.781	2.941	1.00	30.87	C
ATOM	2567	C	ILE	A	706	18.819	-22.337	0.614	1.00	28.18	C
ATOM	2568	O	ILE	A	706	19.894	-21.953	1.060	1.00	27.65	O
ATOM	2569	N	GLU	A	707	18.491	-22.243	-0.671	1.00	28.07	N
ATOM	2571	CA	GLU	A	707	19.419	-21.740	-1.692	1.00	28.72	C
ATOM	2573	CB	GLU	A	707	18.894	-22.020	-3.106	1.00	29.03	C
ATOM	2576	CG	GLU	A	707	18.752	-23.486	-3.462	1.00	31.17	C
ATOM	2579	CD	GLU	A	707	17.385	-24.097	-3.137	1.00	32.70	C
ATOM	2580	OE1	GLU	A	707	16.497	-23.358	-2.611	1.00	31.18	O
ATOM	2581	OE2	GLU	A	707	17.213	-25.329	-3.416	1.00	31.40	O
ATOM	2582	C	GLU	A	707	19.704	-20.229	-1.517	1.00	28.17	C
ATOM	2583	O	GLU	A	707	20.868	-19.790	-1.558	1.00	28.17	O
ATOM	2584	N	GLU	A	708	18.657	-19.443	-1.313	1.00	27.81	N
ATOM	2586	CA	GLU	A	708	18.801	-18.037	-0.910	1.00	28.54	C
ATOM	2588	CB	GLU	A	708	17.451	-17.413	-0.577	1.00	28.31	C
ATOM	2591	CG	GLU	A	708	16.613	-16.992	-1.766	1.00	30.10	C
ATOM	2594	CD	GLU	A	708	15.335	-16.313	-1.319	1.00	29.90	C
ATOM	2595	OE1	GLU	A	708	14.625	-16.911	-0.502	1.00	28.02	O
ATOM	2596	OE2	GLU	A	708	15.061	-15.175	-1.751	1.00	32.16	O
ATOM	2597	C	GLU	A	708	19.665	-17.873	0.348	1.00	28.37	C
ATOM	2598	O	GLU	A	708	20.496	-16.971	0.436	1.00	27.41	O
ATOM	2599	N	TYR	A	709	19.428	-18.739	1.327	1.00	28.74	N
ATOM	2601	CA	TYR	A	709	20.109	-18.658	2.628	1.00	29.07	C
ATOM	2603	CB	TYR	A	709	19.453	-19.633	3.636	1.00	29.15	C
ATOM	2606	CG	TYR	A	709	20.124	-19.744	4.977	1.00	30.08	C
ATOM	2607	CD1	TYR	A	709	19.868	-18.822	5.986	1.00	30.25	C
ATOM	2609	CE1	TYR	A	709	20.487	-18.935	7.224	1.00	32.05	C
ATOM	2611	CZ	TYR	A	709	21.352	-19.981	7.455	1.00	32.81	C
ATOM	2612	OH	TYR	A	709	21.976	-20.115	8.665	1.00	36.64	O
ATOM	2614	CE2	TYR	A	709	21.620	-20.907	6.470	1.00	31.98	C
ATOM	2616	CD2	TYR	A	709	21.002	-20.790	5.248	1.00	30.61	C
ATOM	2618	C	TYR	A	709	21.595	-18.948	2.419	1.00	28.39	C
ATOM	2619	O	TYR	A	709	22.426	-18.153	2.792	1.00	27.85	O
ATOM	2620	N	LYS	A	710	21.924	-20.058	1.773	1.00	29.18	N

ATOM	2622	CA	LYS	A	710	23.326	-20.390	1.544	1.00	30.08	C
ATOM	2624	CB	LYS	A	710	23.482	-21.735	0.879	1.00	30.51	C
ATOM	2627	CG	LYS	A	710	23.191	-22.928	1.801	1.00	32.10	C
ATOM	2630	CD	LYS	A	710	23.256	-24.197	0.984	1.00	34.82	C
ATOM	2633	CE	LYS	A	710	23.178	-25.471	1.823	1.00	36.19	C
ATOM	2636	NZ	LYS	A	710	23.048	-26.659	0.962	1.00	36.81	N
ATOM	2640	C	LYS	A	710	24.077	-19.287	0.758	1.00	30.38	C
ATOM	2641	O	LYS	A	710	25.222	-18.983	1.078	1.00	30.06	O
ATOM	2642	N	THR	A	711	23.419	-18.666	-0.222	1.00	29.86	N
ATOM	2644	CA	THR	A	711	24.062	-17.629	-1.032	1.00	30.01	C
ATOM	2646	CB	THR	A	711	23.217	-17.304	-2.280	1.00	30.66	C
ATOM	2648	OG1	THR	A	711	23.216	-18.436	-3.175	1.00	30.28	O
ATOM	2650	CG2	THR	A	711	23.857	-16.163	-3.073	1.00	30.73	C
ATOM	2654	C	THR	A	711	24.280	-16.369	-0.211	1.00	29.27	C
ATOM	2655	O	THR	A	711	25.323	-15.703	-0.301	1.00	28.15	O
ATOM	2656	N	THR	A	712	23.279	-16.032	0.594	1.00	29.05	N
ATOM	2658	CA	THR	A	712	23.342	-14.843	1.409	1.00	28.20	C
ATOM	2660	CB	THR	A	712	21.986	-14.624	2.128	1.00	28.55	C
ATOM	2662	OG1	THR	A	712	20.947	-14.394	1.162	1.00	26.56	O
ATOM	2664	CG2	THR	A	712	22.014	-13.347	2.972	1.00	28.61	C
ATOM	2668	C	THR	A	712	24.486	-14.963	2.418	1.00	29.25	C
ATOM	2669	O	THR	A	712	25.247	-14.001	2.655	1.00	28.91	O
ATOM	2670	N	LEU	A	713	24.615	-16.130	3.030	1.00	28.84	N
ATOM	2672	CA	LEU	A	713	25.679	-16.335	4.014	1.00	29.18	C
ATOM	2674	CB	LEU	A	713	25.562	-17.704	4.709	1.00	29.57	C
ATOM	2677	CG	LEU	A	713	24.344	-18.001	5.625	1.00	30.84	C
ATOM	2679	CD1	LEU	A	713	24.694	-19.174	6.592	1.00	33.33	C
ATOM	2683	CD2	LEU	A	713	23.816	-16.841	6.360	1.00	32.03	C
ATOM	2687	C	LEU	A	713	27.054	-16.201	3.365	1.00	29.27	C
ATOM	2688	O	LEU	A	713	27.971	-15.605	3.954	1.00	27.98	O
ATOM	2689	N	LYS	A	714	27.203	-16.745	2.162	1.00	28.99	N
ATOM	2691	CA	LYS	A	714	28.458	-16.634	1.431	1.00	29.77	C
ATOM	2693	CB	LYS	A	714	28.397	-17.435	0.129	1.00	30.50	C
ATOM	2696	CG	LYS	A	714	29.555	-17.190	-0.797	1.00	33.47	C
ATOM	2699	CD	LYS	A	714	29.542	-18.049	-2.061	1.00	37.61	C
ATOM	2702	CE	LYS	A	714	28.298	-17.795	-2.928	1.00	41.48	C
ATOM	2705	NZ	LYS	A	714	27.560	-19.063	-3.353	1.00	42.85	N
ATOM	2709	C	LYS	A	714	28.792	-15.172	1.154	1.00	29.88	C
ATOM	2710	O	LYS	A	714	29.921	-14.753	1.379	1.00	30.34	O
ATOM	2711	N	ILE	A	715	27.818	-14.368	0.724	1.00	29.41	N
ATOM	2713	CA	ILE	A	715	28.087	-12.952	0.466	1.00	29.58	C
ATOM	2715	CB	ILE	A	715	26.899	-12.252	-0.272	1.00	29.54	C
ATOM	2717	CG1	ILE	A	715	26.630	-12.898	-1.640	1.00	30.27	C
ATOM	2720	CD1	ILE	A	715	25.236	-12.628	-2.172	1.00	32.00	C
ATOM	2724	CG2	ILE	A	715	27.202	-10.752	-0.452	1.00	30.82	C
ATOM	2728	C	ILE	A	715	28.395	-12.199	1.773	1.00	30.15	C
ATOM	2729	O	ILE	A	715	29.217	-11.283	1.780	1.00	29.32	O
ATOM	2730	N	ILE	A	716	27.695	-12.540	2.865	1.00	30.66	N
ATOM	2732	CA	ILE	A	716	27.948	-11.911	4.159	1.00	30.81	C
ATOM	2734	CB	ILE	A	716	26.953	-12.400	5.284	1.00	30.93	C
ATOM	2736	CG1	ILE	A	716	25.522	-11.939	5.007	1.00	29.96	C
ATOM	2739	CD1	ILE	A	716	24.456	-12.555	5.941	1.00	28.36	C
ATOM	2743	CG2	ILE	A	716	27.377	-11.825	6.666	1.00	32.78	C
ATOM	2747	C	ILE	A	716	29.389	-12.147	4.618	1.00	30.65	C
ATOM	2748	O	ILE	A	716	30.026	-11.204	5.071	1.00	31.73	O
ATOM	2749	N	LYS	A	717	29.861	-13.389	4.530	1.00	31.34	N
ATOM	2751	CA	LYS	A	717	31.224	-13.777	4.906	1.00	32.36	C
ATOM	2753	CB	LYS	A	717	31.507	-15.261	4.657	1.00	32.51	C
ATOM	2756	CG	LYS	A	717	30.760	-16.269	5.531	1.00	35.68	C
ATOM	2759	CD	LYS	A	717	31.032	-17.766	5.106	1.00	38.43	C
ATOM	2762	CE	LYS	A	717	30.092	-18.775	5.818	1.00	39.94	C
ATOM	2765	NZ	LYS	A	717	29.895	-20.095	5.141	1.00	39.44	N
ATOM	2769	C	LYS	A	717	32.239	-12.986	4.095	1.00	32.41	C
ATOM	2770	O	LYS	A	717	33.140	-12.387	4.670	1.00	31.82	O
ATOM	2771	N	GLN	A	718	32.091	-13.001	2.769	1.00	31.49	N
ATOM	2773	CA	GLN	A	718	32.952	-12.216	1.880	1.00	32.27	C
ATOM	2775	CB	GLN	A	718	32.541	-12.381	0.410	1.00	32.06	C

ATOM	2778	CG	GLN	A	718	32.935	-13.729	-0.165	1.00	33.82	C
ATOM	2781	CD	GLN	A	718	32.343	-14.005	-1.544	1.00	36.85	C
ATOM	2782	OE1	GLN	A	718	31.380	-13.347	-1.972	1.00	39.56	O
ATOM	2783	NE2	GLN	A	718	32.923	-14.983	-2.246	1.00	37.92	N
ATOM	2786	C	GLN	A	718	32.958	-10.738	2.257	1.00	31.81	C
ATOM	2787	O	GLN	A	718	34.004	-10.123	2.318	1.00	31.71	O
ATOM	2788	N	ALA	A	719	31.792	-10.182	2.549	1.00	31.68	N
ATOM	2790	CA	ALA	A	719	31.683	-8.762	2.853	1.00	31.63	C
ATOM	2792	CB	ALA	A	719	30.234	-8.325	2.816	1.00	32.00	C
ATOM	2796	C	ALA	A	719	32.312	-8.380	4.189	1.00	32.00	C
ATOM	2797	O	ALA	A	719	32.860	-7.270	4.358	1.00	31.34	O
ATOM	2798	N	ILE	A	720	32.228	-9.284	5.158	1.00	32.32	N
ATOM	2800	CA	ILE	A	720	32.846	-9.021	6.455	1.00	31.42	C
ATOM	2802	CB	ILE	A	720	32.323	-9.983	7.534	1.00	30.69	C
ATOM	2804	CG1	ILE	A	720	30.901	-9.615	7.919	1.00	27.83	C
ATOM	2807	CD1	ILE	A	720	30.313	-10.502	8.957	1.00	27.29	C
ATOM	2811	CG2	ILE	A	720	33.235	-9.947	8.747	1.00	31.46	C
ATOM	2815	C	ILE	A	720	34.354	-9.146	6.316	1.00	31.68	C
ATOM	2816	O	ILE	A	720	35.095	-8.321	6.851	1.00	31.13	O
ATOM	2817	N	LEU	A	721	34.803	-10.186	5.612	1.00	32.18	N
ATOM	2819	CA	LEU	A	721	36.223	-10.374	5.345	1.00	32.91	C
ATOM	2821	CB	LEU	A	721	36.529	-11.711	4.675	1.00	32.99	C
ATOM	2824	CG	LEU	A	721	36.297	-12.971	5.525	1.00	37.70	C
ATOM	2826	CD1	LEU	A	721	36.514	-14.245	4.683	1.00	38.76	C
ATOM	2830	CD2	LEU	A	721	37.162	-13.046	6.745	1.00	40.11	C
ATOM	2834	C	LEU	A	721	36.794	-9.216	4.524	1.00	32.85	C
ATOM	2835	O	LEU	A	721	37.952	-8.836	4.754	1.00	33.57	O
ATOM	2836	N	ALA	A	722	35.986	-8.634	3.630	1.00	31.29	N
ATOM	2838	CA	ALA	A	722	36.363	-7.441	2.873	1.00	31.32	C
ATOM	2840	CB	ALA	A	722	35.201	-6.986	1.947	1.00	31.27	C
ATOM	2844	C	ALA	A	722	36.773	-6.233	3.752	1.00	30.62	C
ATOM	2845	O	ALA	A	722	37.454	-5.358	3.264	1.00	29.59	O
ATOM	2846	N	THR	A	723	36.306	-6.143	5.000	1.00	30.85	N
ATOM	2848	CA	THR	A	723	36.697	-5.038	5.883	1.00	31.23	C
ATOM	2850	CB	THR	A	723	35.712	-4.775	7.046	1.00	30.40	C
ATOM	2852	OG1	THR	A	723	35.606	-5.914	7.911	1.00	32.47	O
ATOM	2854	CG2	THR	A	723	34.344	-4.558	6.528	1.00	32.10	C
ATOM	2858	C	THR	A	723	38.086	-5.173	6.447	1.00	30.74	C
ATOM	2859	O	THR	A	723	38.563	-4.269	7.109	1.00	30.67	O
ATOM	2860	N	ASP	A	724	38.748	-6.280	6.178	1.00	31.33	N
ATOM	2862	CA	ASP	A	724	40.189	-6.380	6.428	1.00	31.24	C
ATOM	2864	CB	ASP	A	724	40.733	-7.764	6.088	1.00	30.66	C
ATOM	2867	CG	ASP	A	724	40.290	-8.837	7.030	1.00	29.75	C
ATOM	2868	OD1	ASP	A	724	39.440	-8.600	7.884	1.00	29.76	O
ATOM	2869	OD2	ASP	A	724	40.762	-9.987	6.987	1.00	30.86	O
ATOM	2870	C	ASP	A	724	40.851	-5.405	5.454	1.00	32.00	C
ATOM	2871	O	ASP	A	724	40.796	-5.626	4.228	1.00	31.56	O
ATOM	2872	N	LEU	A	725	41.451	-4.324	5.949	1.00	30.56	N
ATOM	2874	CA	LEU	A	725	42.126	-3.402	5.030	1.00	30.51	C
ATOM	2876	CB	LEU	A	725	42.814	-2.254	5.771	1.00	31.33	C
ATOM	2879	CG	LEU	A	725	42.583	-0.759	5.485	1.00	34.63	C
ATOM	2881	CD1	LEU	A	725	43.914	-0.011	5.423	1.00	36.43	C
ATOM	2885	CD2	LEU	A	725	41.630	-0.346	4.388	1.00	32.58	C
ATOM	2889	C	LEU	A	725	43.204	-4.053	4.203	1.00	29.44	C
ATOM	2890	O	LEU	A	725	43.438	-3.601	3.108	1.00	29.73	O
ATOM	2891	N	ALA	A	726	43.911	-5.052	4.731	1.00	28.27	N
ATOM	2893	CA	ALA	A	726	44.933	-5.746	3.953	1.00	29.03	C
ATOM	2895	CB	ALA	A	726	45.675	-6.803	4.775	1.00	28.55	C
ATOM	2899	C	ALA	A	726	44.357	-6.370	2.667	1.00	29.70	C
ATOM	2900	O	ALA	A	726	45.053	-6.475	1.677	1.00	28.98	O
ATOM	2901	N	LEU	A	727	43.099	-6.779	2.706	1.00	30.51	N
ATOM	2903	CA	LEU	A	727	42.458	-7.400	1.552	1.00	32.05	C
ATOM	2905	CB	LEU	A	727	41.218	-8.177	1.982	1.00	32.66	C
ATOM	2908	CG	LEU	A	727	41.369	-9.660	2.296	1.00	37.39	C
ATOM	2910	CD1	LEU	A	727	39.942	-10.265	2.429	1.00	39.88	C
ATOM	2914	CD2	LEU	A	727	42.170	-10.453	1.259	1.00	38.45	C
ATOM	2918	C	LEU	A	727	42.044	-6.313	0.546	1.00	30.69	C

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ATOM   2919  O   LEU A 727      42.123  -6.514  -0.637  1.00 29.90           O
ATOM   2920  N   TYR A 728      41.626  -5.166   1.060  1.00 30.15           N
ATOM   2922  CA  TYR A 728      41.334  -4.004   0.242  1.00 29.75           C
ATOM   2924  CB  TYR A 728      40.822  -2.832   1.087  1.00 30.44           C
ATOM   2927  CG  TYR A 728      40.951  -1.498   0.372  1.00 31.40           C
ATOM   2928  CD1 TYR A 728      40.157  -1.192  -0.715  1.00 32.68           C
ATOM   2930  CE1 TYR A 728      40.296   0.029  -1.386  1.00 33.60           C
ATOM   2932  CZ  TYR A 728      41.232   0.939  -0.956  1.00 32.26           C
ATOM   2933  OH  TYR A 728      41.386   2.148  -1.597  1.00 33.27           O
ATOM   2935  CE2 TYR A 728      42.044   0.640   0.111  1.00 32.66           C
ATOM   2937  CD2 TYR A 728      41.888  -0.555   0.782  1.00 31.84           C
ATOM   2939  C   TYR A 728      42.582  -3.607  -0.521  1.00 29.20           C
ATOM   2940  O   TYR A 728      42.541  -3.458  -1.739  1.00 28.20           O
ATOM   2941  N   ILE A 729      43.713  -3.516   0.173  1.00 27.85           N
ATOM   2943  CA  ILE A 729      44.937  -3.036  -0.461  1.00 27.07           C
ATOM   2945  CB  ILE A 729      46.028  -2.741   0.603  1.00 26.18           C
ATOM   2947  CG1 ILE A 729      45.600  -1.552   1.458  1.00 27.32           C
ATOM   2950  CD1 ILE A 729      46.362  -1.426   2.761  1.00 29.88           C
ATOM   2954  CG2 ILE A 729      47.358  -2.423  -0.087  1.00 25.82           C
ATOM   2958  C   ILE A 729      45.428  -4.032  -1.491  1.00 26.41           C
ATOM   2959  O   ILE A 729      45.980  -3.646  -2.520  1.00 25.30           O
ATOM   2960  N   LYS A 730      45.234  -5.309  -1.183  1.00 26.14           N
ATOM   2962  CA  LYS A 730      45.673  -6.381  -2.046  1.00 27.40           C
ATOM   2964  CB  LYS A 730      45.487  -7.734  -1.346  1.00 26.89           C
ATOM   2967  CG  LYS A 730      45.929  -8.938  -2.203  1.00 29.59           C
ATOM   2970  CD  LYS A 730      45.470 -10.258  -1.580  1.00 33.15           C
ATOM   2973  CE  LYS A 730      45.912 -11.470  -2.371  1.00 34.65           C
ATOM   2976  NZ  LYS A 730      45.545 -12.673  -1.583  1.00 38.38           N
ATOM   2980  C   LYS A 730      44.904  -6.360  -3.389  1.00 27.18           C
ATOM   2981  O   LYS A 730      45.489  -6.603  -4.464  1.00 26.31           O
ATOM   2982  N   ARG A 731      43.606  -6.106  -3.316  1.00 27.73           N
ATOM   2984  CA  ARG A 731      42.702  -6.333  -4.469  1.00 29.48           C
ATOM   2986  CB  ARG A 731      41.423  -7.017  -3.999  1.00 29.63           C
ATOM   2989  CG  ARG A 731      41.621  -8.516  -3.662  1.00 34.45           C
ATOM   2992  CD  ARG A 731      40.333  -9.179  -3.157  1.00 39.61           C
ATOM   2995  NE  ARG A 731      39.558  -9.710  -4.292  1.00 45.03           N
ATOM   2997  CZ  ARG A 731      38.213  -9.788  -4.350  1.00 45.41           C
ATOM   2998  NH1 ARG A 731      37.438  -9.436  -3.329  1.00 45.49           N
ATOM   3001  NH2 ARG A 731      37.644 -10.258  -5.447  1.00 47.21           N
ATOM   3004  C   ARG A 731      42.354  -5.067  -5.266  1.00 29.31           C
ATOM   3005  O   ARG A 731      41.908  -5.152  -6.416  1.00 28.60           O
ATOM   3006  N   ARG A 732      42.573  -3.902  -4.661  1.00 29.07           N
ATOM   3008  CA  ARG A 732      42.095  -2.660  -5.242  1.00 29.47           C
ATOM   3010  CB  ARG A 732      42.269  -1.481  -4.300  1.00 29.23           C
ATOM   3013  CG  ARG A 732      43.695  -1.030  -4.066  1.00 29.95           C
ATOM   3016  CD  ARG A 732      43.791   0.013  -2.935  1.00 28.49           C
ATOM   3019  NE  ARG A 732      45.155   0.519  -2.808  1.00 31.38           N
ATOM   3021  CZ  ARG A 732      45.491   1.713  -2.325  1.00 28.56           C
ATOM   3022  NH1 ARG A 732      44.585   2.561  -1.890  1.00 29.84           N
ATOM   3025  NH2 ARG A 732      46.751   2.056  -2.301  1.00 30.13           N
ATOM   3028  C   ARG A 732      42.718  -2.324  -6.580  1.00 30.07           C
ATOM   3029  O   ARG A 732      42.039  -1.750  -7.401  1.00 30.71           O
ATOM   3030  N   GLY A 733      43.987  -2.663  -6.797  1.00 29.98           N
ATOM   3032  CA  GLY A 733      44.666  -2.401  -8.063  1.00 30.89           C
ATOM   3035  C   GLY A 733      43.915  -2.924  -9.281  1.00 32.03           C
ATOM   3036  O   GLY A 733      43.810  -2.237 -10.323  1.00 31.34           O
ATOM   3037  N   GLU A 734      43.384  -4.141  -9.150  1.00 33.29           N
ATOM   3039  CA  GLU A 734      42.573  -4.741 -10.209  1.00 34.28           C
ATOM   3041  CB  GLU A 734      42.197  -6.194  -9.861  1.00 34.83           C
ATOM   3044  CG  GLU A 734      41.219  -6.849 -10.858  1.00 36.43           C
ATOM   3047  CD  GLU A 734      40.859  -8.284 -10.513  1.00 37.93           C
ATOM   3048  OE1 GLU A 734      40.857  -8.644  -9.318  1.00 38.78           O
ATOM   3049  OE2 GLU A 734      40.574  -9.058 -11.458  1.00 41.92           O
ATOM   3050  C   GLU A 734      41.303  -3.904 -10.450  1.00 33.90           C
ATOM   3051  O   GLU A 734      40.929  -3.652 -11.592  1.00 34.11           O
ATOM   3052  N   PHE A 735      40.635  -3.489  -9.374  1.00 34.01           N
ATOM   3054  CA  PHE A 735      39.474  -2.601  -9.476  1.00 33.89           C
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ATOM   3056  CB  PHE A 735      38.942  -2.296  -8.078  1.00 34.08           C
ATOM   3059  CG  PHE A 735      37.713  -1.439  -8.042  1.00 36.84           C
ATOM   3060  CD1 PHE A 735      36.594  -1.745  -8.815  1.00 39.84           C
ATOM   3062  CE1 PHE A 735      35.452  -0.962  -8.756  1.00 40.64           C
ATOM   3064  CZ  PHE A 735      35.407   0.133  -7.910  1.00 41.90           C
ATOM   3066  CE2 PHE A 735      36.512   0.437  -7.105  1.00 40.61           C
ATOM   3068  CD2 PHE A 735      37.650  -0.347  -7.180  1.00 39.10           C
ATOM   3070  C   PHE A 735      39.814  -1.302 -10.191  1.00 33.50           C
ATOM   3071  O   PHE A 735      39.151  -0.934 -11.180  1.00 33.37           O
ATOM   3072  N   PHE A 736      40.844  -0.616  -9.704  1.00 33.05           N
ATOM   3074  CA  PHE A 736      41.217   0.699 -10.206  1.00 34.06           C
ATOM   3076  CB  PHE A 736      42.366   1.295  -9.392  1.00 34.30           C
ATOM   3079  CG  PHE A 736      42.024   1.593  -7.941  1.00 35.16           C
ATOM   3080  CD1 PHE A 736      43.043   1.852  -7.027  1.00 35.56           C
ATOM   3082  CE1 PHE A 736      42.751   2.126  -5.726  1.00 37.00           C
ATOM   3084  CZ  PHE A 736      41.436   2.123  -5.300  1.00 38.26           C
ATOM   3086  CE2 PHE A 736      40.421   1.854  -6.195  1.00 36.37           C
ATOM   3088  CD2 PHE A 736      40.714   1.598  -7.490  1.00 34.99           C
ATOM   3090  C   PHE A 736      41.612   0.665 -11.689  1.00 35.47           C
ATOM   3091  O   PHE A 736      41.337   1.620 -12.416  1.00 35.44           O
ATOM   3092  N   GLU A 737      42.224  -0.447 -12.116  1.00 35.60           N
ATOM   3094  CA  GLU A 737      42.700  -0.632 -13.476  1.00 36.57           C
ATOM   3096  CB  GLU A 737      43.742  -1.765 -13.529  1.00 36.39           C
ATOM   3099  CG  GLU A 737      44.284  -2.096 -14.912  1.00 38.88           C
ATOM   3102  CD  GLU A 737      45.512  -1.281 -15.295  1.00 41.05           C
ATOM   3103  OE1 GLU A 737      45.690  -0.154 -14.778  1.00 42.96           O
ATOM   3104  OE2 GLU A 737      46.300  -1.772 -16.131  1.00 42.28           O
ATOM   3105  C   GLU A 737      41.535  -0.931 -14.421  1.00 36.83           C
ATOM   3106  O   GLU A 737      41.483  -0.395 -15.521  1.00 35.58           O
ATOM   3107  N   LEU A 738      40.615  -1.790 -13.994  1.00 37.36           N
ATOM   3109  CA  LEU A 738      39.409  -2.021 -14.773  1.00 37.62           C
ATOM   3111  CB  LEU A 738      38.474  -3.008 -14.064  1.00 37.25           C
ATOM   3114  CG  LEU A 738      38.872  -4.481 -13.963  1.00 36.35           C
ATOM   3116  CD1 LEU A 738      37.998  -5.192 -12.939  1.00 36.30           C
ATOM   3120  CD2 LEU A 738      38.785  -5.189 -15.300  1.00 37.05           C
ATOM   3124  C   LEU A 738      38.685  -0.688 -15.054  1.00 38.70           C
ATOM   3125  O   LEU A 738      38.291  -0.438 -16.174  1.00 39.15           O
ATOM   3126  N   ILE A 739      38.523   0.172 -14.050  1.00 40.41           N
ATOM   3128  CA  ILE A 739      37.810   1.447 -14.227  1.00 41.58           C
ATOM   3130  CB  ILE A 739      37.419   2.036 -12.858  1.00 41.98           C
ATOM   3132  CG1 ILE A 739      36.243   1.242 -12.282  1.00 41.05           C
ATOM   3135  CD1 ILE A 739      36.094   1.368 -10.859  1.00 41.49           C
ATOM   3139  CG2 ILE A 739      37.024   3.520 -12.973  1.00 42.55           C
ATOM   3143  C   ILE A 739      38.594   2.472 -15.070  1.00 43.09           C
ATOM   3144  O   ILE A 739      38.010   3.155 -15.921  1.00 43.46           O
ATOM   3145  N   ARG A 740      39.902   2.565 -14.828  1.00 43.89           N
ATOM   3147  CA  ARG A 740      40.805   3.523 -15.480  1.00 44.29           C
ATOM   3149  CB  ARG A 740      42.229   3.264 -14.978  1.00 44.62           C
ATOM   3152  CG  ARG A 740      43.352   4.163 -15.486  1.00 45.76           C
ATOM   3155  CD  ARG A 740      44.704   3.845 -14.808  1.00 46.92           C
ATOM   3158  NE  ARG A 740      44.528   3.637 -13.357  1.00 49.06           N
ATOM   3160  CZ  ARG A 740      45.175   2.735 -12.593  1.00 47.38           C
ATOM   3161  NH1 ARG A 740      46.087   1.913 -13.098  1.00 47.75           N
ATOM   3164  NH2 ARG A 740      44.900   2.671 -11.306  1.00 46.41           N
ATOM   3167  C   ARG A 740      40.731   3.329 -16.979  1.00 44.59           C
ATOM   3168  O   ARG A 740      40.689   4.294 -17.759  1.00 44.64           O
ATOM   3169  N   LYS A 741      40.714   2.060 -17.369  1.00 44.87           N
ATOM   3171  CA  LYS A 741      40.325   1.653 -18.706  1.00 45.02           C
ATOM   3173  CB  LYS A 741      40.644   0.170 -18.883  1.00 45.13           C
ATOM   3176  CG  LYS A 741      42.115  -0.177 -18.715  1.00 44.76           C
ATOM   3179  CD  LYS A 741      42.381  -1.583 -19.222  1.00 44.18           C
ATOM   3182  CE  LYS A 741      43.806  -2.043 -18.913  1.00 44.31           C
ATOM   3185  NZ  LYS A 741      44.462  -2.620 -20.128  1.00 43.46           N
ATOM   3189  C   LYS A 741      38.816   1.933 -18.812  1.00 45.27           C
ATOM   3190  O   LYS A 741      38.364   3.001 -18.383  1.00 45.85           O
ATOM   3191  N   ASN A 742      38.028   1.010 -19.358  1.00 44.90           N
ATOM   3193  CA  ASN A 742      36.571   1.065 -19.175  1.00 44.48           C
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ATOM   3195  CB  ASN A 742      35.915   2.030 -20.184  1.00 44.33           C
ATOM   3198  CG  ASN A 742      35.926   3.472 -19.702  1.00 44.37           C
ATOM   3199  OD1 ASN A 742      35.169   3.857 -18.800  1.00 43.50           O
ATOM   3200  ND2 ASN A 742      36.807   4.274 -20.284  1.00 44.38           N
ATOM   3203  C   ASN A 742      36.016  -0.336 -19.333  1.00 44.23           C
ATOM   3204  O   ASN A 742      35.114  -0.581 -20.155  1.00 44.09           O
ATOM   3205  N   GLN A 743      36.598  -1.257 -18.572  1.00 43.32           N
ATOM   3207  CA  GLN A 743      36.328  -2.679 -18.738  1.00 43.18           C
ATOM   3209  CB  GLN A 743      37.643  -3.457 -18.878  1.00 42.99           C
ATOM   3212  CG  GLN A 743      38.443  -3.088 -20.134  1.00 44.00           C
ATOM   3215  CD  GLN A 743      39.778  -3.818 -20.243  1.00 44.62           C
ATOM   3216  OE1 GLN A 743      40.174  -4.554 -19.332  1.00 45.85           O
ATOM   3217  NE2 GLN A 743      40.478  -3.604 -21.351  1.00 44.62           N
ATOM   3220  C   GLN A 743      35.517  -3.216 -17.584  1.00 42.81           C
ATOM   3221  O   GLN A 743      35.214  -4.411 -17.539  1.00 42.59           O
ATOM   3222  N   PHE A 744      35.150  -2.328 -16.657  1.00 43.16           N
ATOM   3224  CA  PHE A 744      34.518  -2.740 -15.422  1.00 42.76           C
ATOM   3226  CB  PHE A 744      34.455  -1.587 -14.429  1.00 43.01           C
ATOM   3229  CG  PHE A 744      33.629  -1.899 -13.220  1.00 43.65           C
ATOM   3230  CD1 PHE A 744      33.922  -3.012 -12.438  1.00 43.33           C
ATOM   3232  CE1 PHE A 744      33.161  -3.319 -11.324  1.00 43.62           C
ATOM   3234  CZ  PHE A 744      32.083  -2.523 -10.987  1.00 44.49           C
ATOM   3236  CE2 PHE A 744      31.767  -1.417 -11.772  1.00 45.02           C
ATOM   3238  CD2 PHE A 744      32.535  -1.115 -12.886  1.00 44.45           C
ATOM   3240  C   PHE A 744      33.123  -3.218 -15.731  1.00 42.74           C
ATOM   3241  O   PHE A 744      32.390  -2.523 -16.412  1.00 42.85           O
ATOM   3242  N   ASN A 745      32.757  -4.384 -15.202  1.00 42.84           N
ATOM   3244  CA  ASN A 745      31.540  -5.082 -15.592  1.00 43.14           C
ATOM   3246  CB  ASN A 745      31.825  -5.887 -16.871  1.00 43.72           C
ATOM   3249  CG  ASN A 745      30.967  -5.444 -18.051  1.00 44.57           C
ATOM   3250  OD1 ASN A 745      29.836  -4.996 -17.874  1.00 47.27           O
ATOM   3251  ND2 ASN A 745      31.506  -5.571 -19.265  1.00 46.68           N
ATOM   3254  C   ASN A 745      30.936  -5.994 -14.498  1.00 43.33           C
ATOM   3255  O   ASN A 745      31.463  -7.082 -14.212  1.00 43.25           O
ATOM   3256  N   LEU A 746      29.817  -5.538 -13.914  1.00 43.48           N
ATOM   3258  CA  LEU A 746      29.080  -6.217 -12.834  1.00 43.30           C
ATOM   3260  CB  LEU A 746      27.926  -5.322 -12.379  1.00 43.40           C
ATOM   3263  CG  LEU A 746      28.272  -4.048 -11.577  1.00 43.36           C
ATOM   3265  CD1 LEU A 746      28.023  -2.727 -12.339  1.00 43.92           C
ATOM   3269  CD2 LEU A 746      27.460  -4.041 -10.334  1.00 43.29           C
ATOM   3273  C   LEU A 746      28.512  -7.603 -13.176  1.00 43.97           C
ATOM   3274  O   LEU A 746      28.046  -8.334 -12.298  1.00 44.14           O
ATOM   3275  N   GLU A 747      28.510  -7.927 -14.462  1.00 44.34           N
ATOM   3277  CA  GLU A 747      28.263  -9.275 -14.966  1.00 44.73           C
ATOM   3279  CB  GLU A 747      28.358  -9.226 -16.491  1.00 44.60           C
ATOM   3282  CG  GLU A 747      29.512  -8.370 -16.999  1.00 44.39           C
ATOM   3285  CD  GLU A 747      30.170  -8.919 -18.260  1.00 45.57           C
ATOM   3286  OE1 GLU A 747      29.449  -9.389 -19.204  1.00 42.97           O
ATOM   3287  OE2 GLU A 747      31.427  -8.864 -18.300  1.00 45.91           O
ATOM   3288  C   GLU A 747      29.154 -10.451 -14.464  1.00 45.39           C
ATOM   3289  O   GLU A 747      28.641 -11.570 -14.274  1.00 45.81           O
ATOM   3290  N   ASP A 748      30.467 -10.252 -14.308  1.00 45.36           N
ATOM   3292  CA  ASP A 748      31.318 -11.384 -13.935  1.00 45.44           C
ATOM   3294  CB  ASP A 748      32.860 -11.184 -14.061  1.00 45.79           C
ATOM   3297  CG  ASP A 748      33.291 -10.175 -15.111  1.00 47.52           C
ATOM   3298  OD1 ASP A 748      34.526 -10.021 -15.246  1.00 50.96           O
ATOM   3299  OD2 ASP A 748      32.531  -9.496 -15.839  1.00 49.90           O
ATOM   3300  C   ASP A 748      31.042 -11.589 -12.460  1.00 44.95           C
ATOM   3301  O   ASP A 748      31.201 -10.649 -11.686  1.00 44.92           O
ATOM   3302  N   PRO A 749      30.710 -12.814 -12.057  1.00 44.38           N
ATOM   3303  CA  PRO A 749      30.700 -13.157 -10.633  1.00 44.02           C
ATOM   3305  CB  PRO A 749      30.837 -14.686 -10.632  1.00 44.29           C
ATOM   3308  CG  PRO A 749      30.281 -15.142 -11.954  1.00 43.95           C
ATOM   3311  CD  PRO A 749      30.386 -13.975 -12.909  1.00 44.40           C
ATOM   3314  C   PRO A 749      31.897 -12.524  -9.906  1.00 43.81           C
ATOM   3315  O   PRO A 749      31.727 -12.026  -8.792  1.00 43.90           O
ATOM   3316  N   HIS A 750      33.068 -12.525 -10.545  1.00 43.08           N
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ATOM   3318  CA  HIS A 750      34.294 -12.034  -9.926  1.00 42.92           C
ATOM   3320  CB  HIS A 750      35.543 -12.469 -10.734  1.00 43.34           C
ATOM   3323  CG  HIS A 750      36.816 -12.062 -10.070  1.00 45.39           C
ATOM   3324  ND1 HIS A 750      37.534 -10.951 -10.458  1.00 46.61           N
ATOM   3326  CE1 HIS A 750      38.566 -10.800  -9.649  1.00 47.97           C
ATOM   3328  NE2 HIS A 750      38.526 -11.750  -8.732  1.00 48.77           N
ATOM   3330  CD2 HIS A 750      37.432 -12.544  -8.964  1.00 48.45           C
ATOM   3332  C   HIS A 750      34.311 -10.509  -9.732  1.00 41.70           C
ATOM   3333  O   HIS A 750      34.591 -10.027  -8.646  1.00 41.56           O
ATOM   3334  N   GLN A 751      34.038  -9.758 -10.791  1.00 40.61           N
ATOM   3336  CA  GLN A 751      34.014  -8.301 -10.705  1.00 39.95           C
ATOM   3338  CB  GLN A 751      33.881  -7.693 -12.098  1.00 39.30           C
ATOM   3341  CG  GLN A 751      35.122  -7.943 -12.951  1.00 38.86           C
ATOM   3344  CD  GLN A 751      35.153  -7.152 -14.235  1.00 36.74           C
ATOM   3345  OE1 GLN A 751      34.985  -5.946 -14.232  1.00 38.17           O
ATOM   3346  NE2 GLN A 751      35.388  -7.826 -15.325  1.00 35.87           N
ATOM   3349  C   GLN A 751      32.914  -7.793  -9.762  1.00 40.04           C
ATOM   3350  O   GLN A 751      33.059  -6.721  -9.140  1.00 40.25           O
ATOM   3351  N   LYS A 752      31.842  -8.581  -9.638  1.00 39.43           N
ATOM   3353  CA  LYS A 752      30.769  -8.341  -8.658  1.00 38.83           C
ATOM   3355  CB  LYS A 752      29.564  -9.302  -8.911  1.00 39.25           C
ATOM   3358  CG  LYS A 752      28.455  -9.313  -7.841  1.00 38.89           C
ATOM   3361  CD  LYS A 752      27.855  -7.931  -7.595  1.00 39.51           C
ATOM   3364  CE  LYS A 752      26.957  -7.457  -8.782  1.00 38.66           C
ATOM   3367  NZ  LYS A 752      25.767  -8.330  -8.981  1.00 38.40           N
ATOM   3371  C   LYS A 752      31.267  -8.492  -7.246  1.00 38.37           C
ATOM   3372  O   LYS A 752      31.015  -7.605  -6.402  1.00 38.69           O
ATOM   3373  N   GLU A 753      31.947  -9.599  -6.947  1.00 38.00           N
ATOM   3375  CA  GLU A 753      32.392  -9.855  -5.574  1.00 39.11           C
ATOM   3377  CB  GLU A 753      33.066 -11.235  -5.422  1.00 40.06           C
ATOM   3380  CG  GLU A 753      32.071 -12.421  -5.462  1.00 43.59           C
ATOM   3383  CD  GLU A 753      32.680 -13.799  -5.799  1.00 48.13           C
ATOM   3384  OE1 GLU A 753      33.907 -13.899  -6.081  1.00 51.83           O
ATOM   3385  OE2 GLU A 753      31.910 -14.806  -5.784  1.00 48.81           O
ATOM   3386  C   GLU A 753      33.328  -8.705  -5.159  1.00 38.39           C
ATOM   3387  O   GLU A 753      33.232  -8.168  -4.074  1.00 37.95           O
ATOM   3388  N   LEU A 754      34.154  -8.304  -6.110  1.00 37.59           N
ATOM   3390  CA  LEU A 754      35.181  -7.295  -5.950  1.00 37.06           C
ATOM   3392  CB  LEU A 754      35.993  -7.307  -7.233  1.00 36.87           C
ATOM   3395  CG  LEU A 754      37.238  -6.492  -7.427  1.00 39.55           C
ATOM   3397  CD1 LEU A 754      36.787  -5.122  -7.839  1.00 42.06           C
ATOM   3401  CD2 LEU A 754      38.169  -6.458  -6.171  1.00 41.44           C
ATOM   3405  C   LEU A 754      34.575  -5.908  -5.693  1.00 36.10           C
ATOM   3406  O   LEU A 754      35.066  -5.140  -4.862  1.00 33.82           O
ATOM   3407  N   PHE A 755      33.517  -5.593  -6.427  1.00 35.18           N
ATOM   3409  CA  PHE A 755      32.812  -4.320  -6.260  1.00 34.80           C
ATOM   3411  CB  PHE A 755      31.773  -4.066  -7.365  1.00 35.11           C
ATOM   3414  CG  PHE A 755      30.958  -2.838  -7.115  1.00 36.68           C
ATOM   3415  CD1 PHE A 755      31.570  -1.598  -7.077  1.00 39.95           C
ATOM   3417  CE1 PHE A 755      30.861  -0.476  -6.794  1.00 41.31           C
ATOM   3419  CZ  PHE A 755      29.522  -0.565  -6.523  1.00 40.36           C
ATOM   3421  CE2 PHE A 755      28.899  -1.780  -6.553  1.00 40.16           C
ATOM   3423  CD2 PHE A 755      29.624  -2.921  -6.821  1.00 39.43           C
ATOM   3425  C   PHE A 755      32.126  -4.275  -4.907  1.00 33.82           C
ATOM   3426  O   PHE A 755      32.186  -3.268  -4.222  1.00 34.54           O
ATOM   3427  N   LEU A 756      31.488  -5.365  -4.510  1.00 33.07           N
ATOM   3429  CA  LEU A 756      30.889  -5.452  -3.190  1.00 33.07           C
ATOM   3431  CB  LEU A 756      30.198  -6.810  -2.988  1.00 33.19           C
ATOM   3434  CG  LEU A 756      28.965  -7.139  -3.870  1.00 35.16           C
ATOM   3436  CD1 LEU A 756      28.482  -8.535  -3.574  1.00 37.25           C
ATOM   3440  CD2 LEU A 756      27.831  -6.214  -3.702  1.00 35.98           C
ATOM   3444  C   LEU A 756      31.937  -5.203  -2.074  1.00 33.15           C
ATOM   3445  O   LEU A 756      31.626  -4.600  -1.017  1.00 31.74           O
ATOM   3446  N   ALA A 757      33.166  -5.682  -2.309  1.00 33.12           N
ATOM   3448  CA  ALA A 757      34.238  -5.537  -1.323  1.00 33.06           C
ATOM   3450  CB  ALA A 757      35.395  -6.482  -1.623  1.00 32.92           C
ATOM   3454  C   ALA A 757      34.712  -4.091  -1.269  1.00 33.03           C
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ATOM	3455	O	ALA	A	757	34.882	-3.542	-0.176	1.00	34.12	O
ATOM	3456	N	MET	A	758	34.864	-3.452	-2.424	1.00	32.95	N
ATOM	3458	CA	MET	A	758	35.271	-2.049	-2.457	1.00	33.93	C
ATOM	3460	CB	MET	A	758	35.527	-1.574	-3.881	1.00	33.91	C
ATOM	3463	CG	MET	A	758	36.672	-2.314	-4.621	1.00	35.83	C
ATOM	3466	SD	MET	A	758	38.384	-2.046	-3.916	1.00	38.11	S
ATOM	3467	CE	MET	A	758	38.783	-3.635	-3.324	1.00	37.96	C
ATOM	3471	C	MET	A	758	34.213	-1.167	-1.771	1.00	34.32	C
ATOM	3472	O	MET	A	758	34.548	-0.214	-1.088	1.00	33.57	O
ATOM	3473	N	LEU	A	759	32.937	-1.517	-1.934	1.00	35.01	N
ATOM	3475	CA	LEU	A	759	31.833	-0.785	-1.311	1.00	35.47	C
ATOM	3477	CB	LEU	A	759	30.519	-1.275	-1.903	1.00	35.61	C
ATOM	3480	CG	LEU	A	759	29.229	-0.558	-1.509	1.00	38.34	C
ATOM	3482	CD1	LEU	A	759	29.323	0.977	-1.635	1.00	38.93	C
ATOM	3486	CD2	LEU	A	759	28.107	-1.116	-2.384	1.00	38.44	C
ATOM	3490	C	LEU	A	759	31.801	-0.916	0.214	1.00	35.04	C
ATOM	3491	O	LEU	A	759	31.481	0.038	0.926	1.00	35.94	O
ATOM	3492	N	MET	A	760	32.084	-2.115	0.715	1.00	34.32	N
ATOM	3494	CA	MET	A	760	32.197	-2.345	2.140	1.00	33.02	C
ATOM	3496	CB	MET	A	760	32.522	-3.821	2.427	1.00	33.34	C
ATOM	3499	CG	MET	A	760	31.323	-4.760	2.332	1.00	32.73	C
ATOM	3502	SD	MET	A	760	29.985	-4.481	3.492	1.00	35.30	S
ATOM	3503	CE	MET	A	760	30.754	-4.506	5.105	1.00	33.09	C
ATOM	3507	C	MET	A	760	33.293	-1.448	2.691	1.00	32.13	C
ATOM	3508	O	MET	A	760	33.105	-0.774	3.689	1.00	31.76	O
ATOM	3509	N	THR	A	761	34.422	-1.403	2.000	1.00	31.96	N
ATOM	3511	CA	THR	A	761	35.526	-0.561	2.431	1.00	31.48	C
ATOM	3513	CB	THR	A	761	36.770	-0.755	1.568	1.00	30.73	C
ATOM	3515	OG1	THR	A	761	37.198	-2.120	1.636	1.00	28.72	O
ATOM	3517	CG2	THR	A	761	37.929	0.055	2.153	1.00	30.78	C
ATOM	3521	C	THR	A	761	35.127	0.897	2.445	1.00	31.80	C
ATOM	3522	O	THR	A	761	35.411	1.597	3.418	1.00	31.67	O
ATOM	3523	N	ALA	A	762	34.438	1.338	1.395	1.00	31.82	N
ATOM	3525	CA	ALA	A	762	34.028	2.740	1.254	1.00	32.31	C
ATOM	3527	CB	ALA	A	762	33.340	2.968	-0.094	1.00	32.30	C
ATOM	3531	C	ALA	A	762	33.110	3.157	2.388	1.00	32.01	C
ATOM	3532	O	ALA	A	762	33.268	4.251	2.954	1.00	32.18	O
ATOM	3533	N	CYS	A	763	32.198	2.256	2.772	1.00	31.80	N
ATOM	3535	CA	CYS	A	763	31.290	2.513	3.913	1.00	31.62	C
ATOM	3537	CB	CYS	A	763	30.127	1.541	3.891	1.00	31.53	C
ATOM	3540	SG	CYS	A	763	29.097	1.770	2.386	1.00	33.88	S
ATOM	3541	C	CYS	A	763	31.986	2.460	5.278	1.00	31.47	C
ATOM	3542	O	CYS	A	763	31.705	3.252	6.142	1.00	32.16	O
ATOM	3543	N	ASP	A	764	32.915	1.529	5.435	1.00	32.05	N
ATOM	3545	CA	ASP	A	764	33.661	1.338	6.657	1.00	32.31	C
ATOM	3547	CB	ASP	A	764	34.548	0.107	6.478	1.00	32.85	C
ATOM	3550	CG	ASP	A	764	34.997	-0.484	7.793	1.00	32.98	C
ATOM	3551	OD1	ASP	A	764	34.339	-0.212	8.822	1.00	35.64	O
ATOM	3552	OD2	ASP	A	764	36.023	-1.205	7.874	1.00	34.26	O
ATOM	3553	C	ASP	A	764	34.536	2.559	7.006	1.00	32.69	C
ATOM	3554	O	ASP	A	764	34.684	2.894	8.176	1.00	32.86	O
ATOM	3555	N	LEU	A	765	35.082	3.221	5.979	1.00	32.87	N
ATOM	3557	CA	LEU	A	765	36.021	4.343	6.156	1.00	33.11	C
ATOM	3559	CB	LEU	A	765	37.121	4.304	5.073	1.00	32.59	C
ATOM	3562	CG	LEU	A	765	37.940	3.020	4.974	1.00	35.12	C
ATOM	3564	CD1	LEU	A	765	39.050	3.150	3.927	1.00	34.93	C
ATOM	3568	CD2	LEU	A	765	38.559	2.603	6.310	1.00	36.80	C
ATOM	3572	C	LEU	A	765	35.337	5.698	6.085	1.00	32.04	C
ATOM	3573	O	LEU	A	765	35.994	6.720	6.171	1.00	31.39	O
ATOM	3574	N	SER	A	766	34.015	5.704	5.940	1.00	32.14	N
ATOM	3576	CA	SER	A	766	33.288	6.902	5.555	1.00	31.82	C
ATOM	3578	CB	SER	A	766	31.860	6.549	5.094	1.00	32.63	C
ATOM	3581	OG	SER	A	766	31.110	5.983	6.153	1.00	32.46	O
ATOM	3583	C	SER	A	766	33.230	8.033	6.590	1.00	31.12	C
ATOM	3584	O	SER	A	766	32.846	9.120	6.226	1.00	30.64	O
ATOM	3585	N	ALA	A	767	33.600	7.802	7.851	1.00	30.80	N
ATOM	3587	CA	ALA	A	767	33.854	8.938	8.767	1.00	30.32	C

ATOM	3589	CB	ALA	A	767	34.492	8.458	10.078	1.00	30.27	C
ATOM	3593	C	ALA	A	767	34.763	9.988	8.092	1.00	29.89	C
ATOM	3594	O	ALA	A	767	34.628	11.170	8.343	1.00	29.21	O
ATOM	3595	N	ILE	A	768	35.694	9.538	7.239	1.00	30.00	N
ATOM	3597	CA	ILE	A	768	36.646	10.422	6.569	1.00	30.23	C
ATOM	3599	CB	ILE	A	768	37.817	9.582	5.948	1.00	30.76	C
ATOM	3601	CG1	ILE	A	768	39.050	10.423	5.684	1.00	32.05	C
ATOM	3604	CD1	ILE	A	768	39.698	11.003	6.933	1.00	35.25	C
ATOM	3608	CG2	ILE	A	768	37.413	9.018	4.601	1.00	32.10	C
ATOM	3612	C	ILE	A	768	35.999	11.349	5.524	1.00	30.34	C
ATOM	3613	O	ILE	A	768	36.625	12.337	5.070	1.00	30.09	O
ATOM	3614	N	THR	A	769	34.770	11.048	5.125	1.00	30.53	N
ATOM	3616	CA	THR	A	769	34.037	11.910	4.186	1.00	31.36	C
ATOM	3618	CB	THR	A	769	33.212	11.057	3.209	1.00	31.66	C
ATOM	3620	OG1	THR	A	769	32.133	10.411	3.905	1.00	31.66	O
ATOM	3622	CG2	THR	A	769	34.031	9.921	2.660	1.00	31.10	C
ATOM	3626	C	THR	A	769	33.087	12.926	4.840	1.00	31.89	C
ATOM	3627	O	THR	A	769	32.474	13.732	4.144	1.00	32.05	O
ATOM	3628	N	LYS	A	770	32.976	12.908	6.164	1.00	31.96	N
ATOM	3630	CA	LYS	A	770	31.939	13.694	6.859	1.00	31.48	C
ATOM	3632	CB	LYS	A	770	31.757	13.185	8.300	1.00	31.33	C
ATOM	3635	CG	LYS	A	770	31.213	11.754	8.406	1.00	31.96	C
ATOM	3638	CD	LYS	A	770	29.743	11.641	8.063	1.00	33.71	C
ATOM	3641	CE	LYS	A	770	29.300	10.166	8.044	1.00	35.91	C
ATOM	3644	NZ	LYS	A	770	27.964	9.987	7.459	1.00	35.13	N
ATOM	3648	C	LYS	A	770	32.251	15.172	6.867	1.00	30.72	C
ATOM	3649	O	LYS	A	770	33.400	15.549	6.735	1.00	31.50	O
ATOM	3650	N	PRO	A	771	31.243	16.034	7.036	1.00	30.88	N
ATOM	3651	CA	PRO	A	771	31.515	17.472	7.157	1.00	30.34	C
ATOM	3653	CB	PRO	A	771	30.162	18.066	7.577	1.00	30.70	C
ATOM	3656	CG	PRO	A	771	29.144	17.093	7.075	1.00	29.87	C
ATOM	3659	CD	PRO	A	771	29.801	15.730	7.154	1.00	30.49	C
ATOM	3662	C	PRO	A	771	32.578	17.678	8.234	1.00	30.56	C
ATOM	3663	O	PRO	A	771	32.621	16.901	9.192	1.00	30.01	O
ATOM	3664	N	TRP	A	772	33.415	18.690	8.067	1.00	30.74	N
ATOM	3666	CA	TRP	A	772	34.580	18.907	8.922	1.00	31.54	C
ATOM	3668	CB	TRP	A	772	35.215	20.260	8.605	1.00	31.15	C
ATOM	3671	CG	TRP	A	772	36.310	20.628	9.554	1.00	31.74	C
ATOM	3672	CD1	TRP	A	772	36.346	21.706	10.395	1.00	30.69	C
ATOM	3674	NE1	TRP	A	772	37.514	21.708	11.119	1.00	30.64	N
ATOM	3676	CE2	TRP	A	772	38.252	20.610	10.766	1.00	32.70	C
ATOM	3677	CD2	TRP	A	772	37.525	19.911	9.777	1.00	31.92	C
ATOM	3678	CE3	TRP	A	772	38.075	18.741	9.247	1.00	33.06	C
ATOM	3680	CZ3	TRP	A	772	39.310	18.315	9.700	1.00	32.82	C
ATOM	3682	CH2	TRP	A	772	40.017	19.042	10.664	1.00	33.20	C
ATOM	3684	CZ2	TRP	A	772	39.505	20.191	11.210	1.00	32.99	C
ATOM	3686	C	TRP	A	772	34.370	18.772	10.448	1.00	32.29	C
ATOM	3687	O	TRP	A	772	35.158	18.085	11.082	1.00	32.63	O
ATOM	3688	N	PRO	A	773	33.353	19.406	11.045	1.00	32.84	N
ATOM	3689	CA	PRO	A	773	33.174	19.318	12.505	1.00	33.39	C
ATOM	3691	CB	PRO	A	773	31.938	20.202	12.785	1.00	33.59	C
ATOM	3694	CG	PRO	A	773	31.831	21.109	11.595	1.00	33.72	C
ATOM	3697	CD	PRO	A	773	32.313	20.241	10.420	1.00	32.94	C
ATOM	3700	C	PRO	A	773	32.932	17.897	13.000	1.00	33.89	C
ATOM	3701	O	PRO	A	773	33.352	17.574	14.108	1.00	34.70	O
ATOM	3702	N	ILE	A	774	32.275	17.069	12.200	1.00	33.06	N
ATOM	3704	CA	ILE	A	774	32.049	15.689	12.561	1.00	33.95	C
ATOM	3706	CB	ILE	A	774	30.906	15.131	11.696	1.00	34.28	C
ATOM	3708	CG1	ILE	A	774	29.592	15.855	12.055	1.00	36.56	C
ATOM	3711	CD1	ILE	A	774	28.580	15.882	10.928	1.00	37.65	C
ATOM	3715	CG2	ILE	A	774	30.780	13.606	11.848	1.00	34.99	C
ATOM	3719	C	ILE	A	774	33.317	14.831	12.421	1.00	34.03	C
ATOM	3720	O	ILE	A	774	33.637	14.032	13.311	1.00	34.05	O
ATOM	3721	N	GLN	A	775	34.029	14.991	11.305	1.00	33.53	N
ATOM	3723	CA	GLN	A	775	35.276	14.259	11.075	1.00	33.26	C
ATOM	3725	CB	GLN	A	775	35.781	14.483	9.630	1.00	32.88	C
ATOM	3728	CG	GLN	A	775	37.294	14.320	9.411	1.00	32.21	C

ATOM	3731	CD	GLN	A	775	37.819	12.966	9.789	1.00	29.57	C
ATOM	3732	OE1	GLN	A	775	37.072	11.968	9.828	1.00	32.23	O
ATOM	3733	NE2	GLN	A	775	39.108	12.904	10.058	1.00	27.57	N
ATOM	3736	C	GLN	A	775	36.334	14.620	12.138	1.00	33.48	C
ATOM	3737	O	GLN	A	775	36.931	13.721	12.725	1.00	33.59	O
ATOM	3738	N	GLN	A	776	36.553	15.912	12.389	1.00	33.83	N
ATOM	3740	CA	GLN	A	776	37.544	16.350	13.391	1.00	34.39	C
ATOM	3742	CB	GLN	A	776	37.540	17.866	13.599	1.00	34.52	C
ATOM	3745	CG	GLN	A	776	38.810	18.396	14.333	1.00	35.28	C
ATOM	3748	CD	GLN	A	776	38.898	19.924	14.435	1.00	35.65	C
ATOM	3749	OE1	GLN	A	776	39.982	20.490	14.355	1.00	35.83	O
ATOM	3750	NE2	GLN	A	776	37.767	20.577	14.641	1.00	37.48	N
ATOM	3753	C	GLN	A	776	37.254	15.648	14.709	1.00	35.11	C
ATOM	3754	O	GLN	A	776	38.138	15.040	15.306	1.00	34.23	O
ATOM	3755	N	ARG	A	777	35.989	15.710	15.120	1.00	35.48	N
ATOM	3757	CA	ARG	A	777	35.524	15.082	16.349	1.00	36.93	C
ATOM	3759	CB	ARG	A	777	34.033	15.360	16.534	1.00	37.68	C
ATOM	3762	CG	ARG	A	777	33.637	15.541	17.927	1.00	42.37	C
ATOM	3765	CD	ARG	A	777	33.662	14.250	18.764	1.00	47.31	C
ATOM	3768	NE	ARG	A	777	33.889	14.588	20.177	1.00	50.43	N
ATOM	3770	CZ	ARG	A	777	32.935	14.780	21.062	1.00	49.65	C
ATOM	3771	NH1	ARG	A	777	31.654	14.659	20.708	1.00	51.41	N
ATOM	3774	NH2	ARG	A	777	33.270	15.086	22.304	1.00	49.88	N
ATOM	3777	C	ARG	A	777	35.747	13.568	16.411	1.00	36.41	C
ATOM	3778	O	ARG	A	777	36.304	13.087	17.386	1.00	36.09	O
ATOM	3779	N	ILE	A	778	35.291	12.826	15.401	1.00	35.82	N
ATOM	3781	CA	ILE	A	778	35.476	11.377	15.379	1.00	36.75	C
ATOM	3783	CB	ILE	A	778	34.957	10.723	14.057	1.00	36.93	C
ATOM	3785	CG1	ILE	A	778	33.437	10.792	13.978	1.00	39.16	C
ATOM	3788	CD1	ILE	A	778	32.868	10.202	12.692	1.00	40.52	C
ATOM	3792	CG2	ILE	A	778	35.355	9.247	14.011	1.00	39.29	C
ATOM	3796	C	ILE	A	778	36.944	11.027	15.497	1.00	35.85	C
ATOM	3797	O	ILE	A	778	37.312	10.105	16.244	1.00	35.82	O
ATOM	3798	N	ALA	A	779	37.765	11.754	14.730	1.00	34.32	N
ATOM	3800	CA	ALA	A	779	39.186	11.455	14.599	1.00	33.17	C
ATOM	3802	CB	ALA	A	779	39.824	12.324	13.505	1.00	32.80	C
ATOM	3806	C	ALA	A	779	39.854	11.697	15.947	1.00	32.84	C
ATOM	3807	O	ALA	A	779	40.703	10.928	16.366	1.00	31.43	O
ATOM	3808	N	GLU	A	780	39.430	12.750	16.645	1.00	32.74	N
ATOM	3810	CA	GLU	A	780	39.957	13.022	17.982	1.00	33.52	C
ATOM	3812	CB	GLU	A	780	39.479	14.371	18.519	1.00	34.36	C
ATOM	3815	CG	GLU	A	780	39.675	15.507	17.542	1.00	37.36	C
ATOM	3818	CD	GLU	A	780	40.731	16.458	17.945	1.00	42.43	C
ATOM	3819	OE1	GLU	A	780	40.376	17.546	18.475	1.00	44.44	O
ATOM	3820	OE2	GLU	A	780	41.913	16.111	17.675	1.00	47.53	O
ATOM	3821	C	GLU	A	780	39.587	11.965	18.986	1.00	32.66	C
ATOM	3822	O	GLU	A	780	40.396	11.621	19.829	1.00	32.54	O
ATOM	3823	N	LEU	A	781	38.360	11.471	18.904	1.00	32.94	N
ATOM	3825	CA	LEU	A	781	37.849	10.459	19.841	1.00	33.38	C
ATOM	3827	CB	LEU	A	781	36.356	10.264	19.662	1.00	33.14	C
ATOM	3830	CG	LEU	A	781	35.463	11.268	20.393	1.00	37.00	C
ATOM	3832	CD1	LEU	A	781	34.009	10.910	20.097	1.00	40.21	C
ATOM	3836	CD2	LEU	A	781	35.731	11.316	21.899	1.00	37.56	C
ATOM	3840	C	LEU	A	781	38.558	9.140	19.623	1.00	33.33	C
ATOM	3841	O	LEU	A	781	39.014	8.479	20.568	1.00	33.55	O
ATOM	3842	N	VAL	A	782	38.689	8.810	18.354	1.00	33.23	N
ATOM	3844	CA	VAL	A	782	39.315	7.586	17.916	1.00	34.70	C
ATOM	3846	CB	VAL	A	782	39.143	7.435	16.382	1.00	35.07	C
ATOM	3848	CG1	VAL	A	782	40.142	6.493	15.802	1.00	37.35	C
ATOM	3852	CG2	VAL	A	782	37.706	7.020	16.073	1.00	34.78	C
ATOM	3856	C	VAL	A	782	40.758	7.578	18.352	1.00	34.45	C
ATOM	3857	O	VAL	A	782	41.237	6.597	18.933	1.00	35.02	O
ATOM	3858	N	ALA	A	783	41.425	8.711	18.158	1.00	34.54	N
ATOM	3860	CA	ALA	A	783	42.802	8.854	18.570	1.00	34.07	C
ATOM	3862	CB	ALA	A	783	43.366	10.195	18.084	1.00	34.19	C
ATOM	3866	C	ALA	A	783	42.940	8.715	20.077	1.00	33.96	C
ATOM	3867	O	ALA	A	783	43.844	8.055	20.563	1.00	34.29	O

ATOM	3868	N	THR	A	784	42.061	9.362	20.829	1.00	33.54	N
ATOM	3870	CA	THR	A	784	42.079	9.254	22.284	1.00	33.02	C
ATOM	3872	CB	THR	A	784	40.985	10.163	22.882	1.00	33.03	C
ATOM	3874	OG1	THR	A	784	41.372	11.520	22.687	1.00	33.63	O
ATOM	3876	CG2	THR	A	784	40.873	9.995	24.372	1.00	34.14	C
ATOM	3880	C	THR	A	784	41.836	7.808	22.713	1.00	32.12	C
ATOM	3881	O	THR	A	784	42.523	7.293	23.585	1.00	30.96	O
ATOM	3882	N	GLU	A	785	40.881	7.150	22.073	1.00	31.76	N
ATOM	3884	CA	GLU	A	785	40.597	5.763	22.421	1.00	32.72	C
ATOM	3886	CB	GLU	A	785	39.424	5.240	21.629	1.00	33.09	C
ATOM	3889	CG	GLU	A	785	38.120	5.797	22.173	1.00	35.55	C
ATOM	3892	CD	GLU	A	785	37.114	6.142	21.097	1.00	39.51	C
ATOM	3893	OE1	GLU	A	785	37.160	5.569	19.956	1.00	37.48	O
ATOM	3894	OE2	GLU	A	785	36.253	6.992	21.418	1.00	40.23	O
ATOM	3895	C	GLU	A	785	41.823	4.862	22.270	1.00	32.40	C
ATOM	3896	O	GLU	A	785	42.063	4.000	23.121	1.00	32.01	O
ATOM	3897	N	PHE	A	786	42.637	5.090	21.236	1.00	31.75	N
ATOM	3899	CA	PHE	A	786	43.821	4.266	21.052	1.00	31.08	C
ATOM	3901	CB	PHE	A	786	44.302	4.254	19.589	1.00	30.76	C
ATOM	3904	CG	PHE	A	786	45.638	3.603	19.430	1.00	28.95	C
ATOM	3905	CD1	PHE	A	786	45.733	2.227	19.231	1.00	28.19	C
ATOM	3907	CE1	PHE	A	786	46.966	1.598	19.126	1.00	26.34	C
ATOM	3909	CZ	PHE	A	786	48.124	2.354	19.193	1.00	28.48	C
ATOM	3911	CE2	PHE	A	786	48.046	3.737	19.396	1.00	29.04	C
ATOM	3913	CD2	PHE	A	786	46.800	4.352	19.506	1.00	27.06	C
ATOM	3915	C	PHE	A	786	44.954	4.709	21.954	1.00	31.42	C
ATOM	3916	O	PHE	A	786	45.556	3.899	22.664	1.00	31.10	O
ATOM	3917	N	PHE	A	787	45.274	5.997	21.904	1.00	31.90	N
ATOM	3919	CA	PHE	A	787	46.485	6.498	22.527	1.00	33.00	C
ATOM	3921	CB	PHE	A	787	46.832	7.920	22.033	1.00	32.94	C
ATOM	3924	CG	PHE	A	787	47.416	7.944	20.647	1.00	33.16	C
ATOM	3925	CD1	PHE	A	787	46.739	8.554	19.592	1.00	34.71	C
ATOM	3927	CE1	PHE	A	787	47.260	8.551	18.300	1.00	34.34	C
ATOM	3929	CZ	PHE	A	787	48.445	7.937	18.057	1.00	32.95	C
ATOM	3931	CE2	PHE	A	787	49.141	7.319	19.119	1.00	33.74	C
ATOM	3933	CD2	PHE	A	787	48.616	7.315	20.387	1.00	31.79	C
ATOM	3935	C	PHE	A	787	46.451	6.431	24.058	1.00	34.24	C
ATOM	3936	O	PHE	A	787	47.504	6.333	24.660	1.00	33.67	O
ATOM	3937	N	ASP	A	788	45.261	6.426	24.659	1.00	35.47	N
ATOM	3939	CA	ASP	A	788	45.120	6.375	26.118	1.00	37.43	C
ATOM	3941	CB	ASP	A	788	44.295	7.571	26.622	1.00	37.71	C
ATOM	3944	CG	ASP	A	788	44.916	8.910	26.261	1.00	39.74	C
ATOM	3945	OD1	ASP	A	788	46.162	9.028	26.237	1.00	41.13	O
ATOM	3946	OD2	ASP	A	788	44.226	9.908	25.985	1.00	44.69	O
ATOM	3947	C	ASP	A	788	44.430	5.097	26.586	1.00	38.24	C
ATOM	3948	O	ASP	A	788	43.745	5.111	27.604	1.00	39.37	O
ATOM	3949	N	GLN	A	789	44.604	3.998	25.856	1.00	38.19	N
ATOM	3951	CA	GLN	A	789	43.942	2.744	26.211	1.00	38.18	C
ATOM	3953	CB	GLN	A	789	43.888	1.806	24.985	1.00	37.38	C
ATOM	3956	CG	GLN	A	789	45.238	1.289	24.523	1.00	36.46	C
ATOM	3959	CD	GLN	A	789	45.095	0.409	23.304	1.00	36.18	C
ATOM	3960	OE1	GLN	A	789	45.349	0.839	22.152	1.00	34.24	O
ATOM	3961	NE2	GLN	A	789	44.654	-0.815	23.536	1.00	30.76	N
ATOM	3964	C	GLN	A	789	44.563	2.029	27.439	1.00	38.14	C
ATOM	3965	O	GLN	A	789	45.646	2.404	27.938	1.00	39.39	O
ATOM	3966	N	LEU	A	804	47.292	14.102	30.667	1.00	48.20	N
ATOM	3968	CA	LEU	A	804	48.025	15.289	31.085	1.00	48.23	C
ATOM	3970	CB	LEU	A	804	48.616	15.100	32.503	1.00	48.00	C
ATOM	3973	CG	LEU	A	804	47.806	15.491	33.769	1.00	46.92	C
ATOM	3975	CD1	LEU	A	804	48.696	15.513	35.023	1.00	45.99	C
ATOM	3979	CD2	LEU	A	804	47.075	16.830	33.646	1.00	46.22	C
ATOM	3983	C	LEU	A	804	49.131	15.673	30.083	1.00	48.83	C
ATOM	3984	O	LEU	A	804	50.011	16.473	30.414	1.00	48.85	O
ATOM	3985	N	MET	A	805	49.098	15.113	28.869	1.00	49.61	N
ATOM	3987	CA	MET	A	805	49.982	15.606	27.787	1.00	50.21	C
ATOM	3989	CB	MET	A	805	50.983	14.551	27.265	1.00	50.52	C
ATOM	3992	CG	MET	A	805	50.399	13.177	26.872	1.00	52.14	C

ATOM	3995	SD	MET	A	805	51.017	11.863	27.954	1.00	55.69	S
ATOM	3996	CE	MET	A	805	52.683	11.540	27.248	1.00	53.87	C
ATOM	4000	C	MET	A	805	49.149	16.213	26.649	1.00	49.81	C
ATOM	4001	O	MET	A	805	49.319	17.404	26.356	1.00	50.89	O
ATOM	4002	N	ASN	A	806	48.271	15.410	26.030	1.00	48.96	N
ATOM	4004	CA	ASN	A	806	47.228	15.890	25.111	1.00	48.12	C
ATOM	4006	CB	ASN	A	806	46.341	16.948	25.794	1.00	48.40	C
ATOM	4009	CG	ASN	A	806	45.306	16.328	26.687	1.00	48.99	C
ATOM	4010	OD1	ASN	A	806	44.550	15.469	26.243	1.00	48.89	O
ATOM	4011	ND2	ASN	A	806	45.286	16.730	27.963	1.00	48.58	N
ATOM	4014	C	ASN	A	806	47.739	16.454	23.804	1.00	46.98	C
ATOM	4015	O	ASN	A	806	47.348	15.982	22.743	1.00	46.74	O
ATOM	4016	N	ARG	A	807	48.504	17.540	23.912	1.00	45.71	N
ATOM	4018	CA	ARG	A	807	49.315	18.096	22.837	1.00	44.96	C
ATOM	4020	CB	ARG	A	807	50.422	18.984	23.418	1.00	44.75	C
ATOM	4023	CG	ARG	A	807	49.988	20.372	23.859	1.00	44.98	C
ATOM	4026	CD	ARG	A	807	51.038	21.442	23.571	1.00	44.24	C
ATOM	4029	NE	ARG	A	807	51.043	22.518	24.553	1.00	44.44	N
ATOM	4031	CZ	ARG	A	807	51.802	23.608	24.469	1.00	44.47	C
ATOM	4032	NH1	ARG	A	807	52.636	23.781	23.445	1.00	44.73	N
ATOM	4035	NH2	ARG	A	807	51.736	24.535	25.419	1.00	44.86	N
ATOM	4038	C	ARG	A	807	49.977	17.018	22.007	1.00	44.15	C
ATOM	4039	O	ARG	A	807	49.721	16.930	20.817	1.00	44.46	O
ATOM	4040	N	GLU	A	808	50.823	16.206	22.640	1.00	43.47	N
ATOM	4042	CA	GLU	A	808	51.549	15.127	21.952	1.00	43.32	C
ATOM	4044	CB	GLU	A	808	52.339	14.273	22.962	1.00	43.39	C
ATOM	4047	CG	GLU	A	808	53.743	14.811	23.263	1.00	44.43	C
ATOM	4050	CD	GLU	A	808	54.087	14.859	24.752	1.00	46.76	C
ATOM	4051	OE1	GLU	A	808	53.612	13.988	25.518	1.00	49.69	O
ATOM	4052	OE2	GLU	A	808	54.847	15.768	25.166	1.00	46.32	O
ATOM	4053	C	GLU	A	808	50.621	14.252	21.100	1.00	42.71	C
ATOM	4054	O	GLU	A	808	50.993	13.807	20.015	1.00	43.71	O
ATOM	4055	N	LYS	A	809	49.411	14.028	21.591	1.00	41.53	N
ATOM	4057	CA	LYS	A	809	48.393	13.268	20.879	1.00	41.25	C
ATOM	4059	CB	LYS	A	809	47.240	12.930	21.861	1.00	41.18	C
ATOM	4062	CG	LYS	A	809	46.242	11.871	21.390	1.00	40.13	C
ATOM	4065	CD	LYS	A	809	44.800	12.047	21.921	1.00	39.55	C
ATOM	4068	CE	LYS	A	809	44.702	12.763	23.279	1.00	41.48	C
ATOM	4071	NZ	LYS	A	809	43.352	12.612	23.881	1.00	42.05	N
ATOM	4075	C	LYS	A	809	47.854	14.078	19.692	1.00	40.56	C
ATOM	4076	O	LYS	A	809	47.896	13.638	18.547	1.00	40.59	O
ATOM	4077	N	LYS	A	810	47.353	15.270	19.996	1.00	39.94	N
ATOM	4079	CA	LYS	A	810	46.595	16.086	19.056	1.00	39.38	C
ATOM	4081	CB	LYS	A	810	46.030	17.334	19.750	1.00	39.37	C
ATOM	4084	CG	LYS	A	810	44.595	17.168	20.259	1.00	41.26	C
ATOM	4087	CD	LYS	A	810	44.144	18.418	21.043	1.00	43.79	C
ATOM	4090	CE	LYS	A	810	42.631	18.636	20.928	1.00	44.96	C
ATOM	4093	NZ	LYS	A	810	42.290	20.033	21.357	1.00	46.60	N
ATOM	4097	C	LYS	A	810	47.447	16.501	17.871	1.00	38.49	C
ATOM	4098	O	LYS	A	810	46.935	16.581	16.755	1.00	38.38	O
ATOM	4099	N	ASN	A	811	48.728	16.756	18.138	1.00	37.29	N
ATOM	4101	CA	ASN	A	811	49.707	17.123	17.120	1.00	37.08	C
ATOM	4103	CB	ASN	A	811	51.015	17.581	17.771	1.00	36.70	C
ATOM	4106	CG	ASN	A	811	50.949	19.013	18.267	1.00	38.21	C
ATOM	4107	OD1	ASN	A	811	50.010	19.751	17.962	1.00	40.42	O
ATOM	4108	ND2	ASN	A	811	51.951	19.415	19.039	1.00	36.98	N
ATOM	4111	C	ASN	A	811	50.029	16.029	16.094	1.00	36.40	C
ATOM	4112	O	ASN	A	811	50.519	16.338	15.020	1.00	36.71	O
ATOM	4113	N	LYS	A	812	49.794	14.765	16.443	1.00	35.59	N
ATOM	4115	CA	LYS	A	812	49.952	13.658	15.515	1.00	34.80	C
ATOM	4117	CB	LYS	A	812	49.997	12.321	16.261	1.00	35.36	C
ATOM	4120	CG	LYS	A	812	51.178	12.089	17.197	1.00	38.10	C
ATOM	4123	CD	LYS	A	812	50.943	10.811	18.045	1.00	40.15	C
ATOM	4126	CE	LYS	A	812	52.167	10.495	18.955	1.00	42.19	C
ATOM	4129	NZ	LYS	A	812	51.729	10.181	20.375	1.00	43.84	N
ATOM	4133	C	LYS	A	812	48.809	13.565	14.505	1.00	33.50	C
ATOM	4134	O	LYS	A	812	49.002	13.066	13.399	1.00	33.42	O

ATOM	4135	N	ILE	A	813	47.612	13.997	14.885	1.00 31.89	N
ATOM	4137	CA	ILE	A	813	46.419	13.628	14.139	1.00 31.20	C
ATOM	4139	CB	ILE	A	813	45.112	13.971	14.923	1.00 31.60	C
ATOM	4141	CG1	ILE	A	813	45.053	13.185	16.235	1.00 31.56	C
ATOM	4144	CD1	ILE	A	813	43.972	13.688	17.154	1.00 33.08	C
ATOM	4148	CG2	ILE	A	813	43.856	13.633	14.078	1.00 31.70	C
ATOM	4152	C	ILE	A	813	46.335	14.128	12.685	1.00 30.20	C
ATOM	4153	O	ILE	A	813	45.916	13.360	11.822	1.00 29.75	O
ATOM	4154	N	PRO	A	814	46.641	15.400	12.420	1.00 29.48	N
ATOM	4155	CA	PRO	A	814	46.591	15.922	11.047	1.00 29.05	C
ATOM	4157	CB	PRO	A	814	47.109	17.351	11.195	1.00 29.41	C
ATOM	4160	CG	PRO	A	814	46.719	17.725	12.636	1.00 28.96	C
ATOM	4163	CD	PRO	A	814	46.977	16.463	13.391	1.00 29.10	C
ATOM	4166	C	PRO	A	814	47.414	15.109	10.049	1.00 28.97	C
ATOM	4167	O	PRO	A	814	46.860	14.740	9.019	1.00 28.23	O
ATOM	4168	N	SER	A	815	48.653	14.778	10.377	1.00 28.56	N
ATOM	4170	CA	SER	A	815	49.506	14.002	9.462	1.00 29.09	C
ATOM	4172	CB	SER	A	815	50.962	13.975	9.927	1.00 28.44	C
ATOM	4175	OG	SER	A	815	51.511	15.248	9.770	1.00 32.41	O
ATOM	4177	C	SER	A	815	49.001	12.586	9.329	1.00 28.45	C
ATOM	4178	O	SER	A	815	49.115	12.000	8.246	1.00 27.40	O
ATOM	4179	N	MET	A	816	48.443	12.039	10.417	1.00 27.90	N
ATOM	4181	CA	MET	A	816	47.820	10.719	10.346	1.00 27.88	C
ATOM	4183	CB	MET	A	816	47.290	10.260	11.710	1.00 27.50	C
ATOM	4186	CG	MET	A	816	48.410	10.004	12.750	1.00 31.73	C
ATOM	4189	SD	MET	A	816	47.733	9.786	14.449	1.00 35.38	S
ATOM	4190	CE	MET	A	816	47.038	8.358	14.309	1.00 33.86	C
ATOM	4194	C	MET	A	816	46.685	10.684	9.332	1.00 27.46	C
ATOM	4195	O	MET	A	816	46.606	9.769	8.502	1.00 26.95	O
ATOM	4196	N	GLN	A	817	45.791	11.654	9.418	1.00 27.39	N
ATOM	4198	CA	GLN	A	817	44.628	11.668	8.558	1.00 27.92	C
ATOM	4200	CB	GLN	A	817	43.554	12.624	9.076	1.00 27.63	C
ATOM	4203	CG	GLN	A	817	42.950	12.260	10.414	1.00 29.16	C
ATOM	4206	CD	GLN	A	817	42.262	10.929	10.457	1.00 31.41	C
ATOM	4207	OE1	GLN	A	817	41.024	10.843	10.344	1.00 36.06	O
ATOM	4208	NE2	GLN	A	817	43.035	9.881	10.652	1.00 31.67	N
ATOM	4211	C	GLN	A	817	45.011	11.996	7.108	1.00 27.83	C
ATOM	4212	O	GLN	A	817	44.525	11.359	6.165	1.00 27.95	O
ATOM	4213	N	VAL	A	818	45.888	12.958	6.917	1.00 28.09	N
ATOM	4215	CA	VAL	A	818	46.317	13.248	5.562	1.00 28.52	C
ATOM	4217	CB	VAL	A	818	47.221	14.452	5.472	1.00 28.62	C
ATOM	4219	CG1	VAL	A	818	47.834	14.545	4.071	1.00 29.96	C
ATOM	4223	CG2	VAL	A	818	46.447	15.702	5.757	1.00 29.17	C
ATOM	4227	C	VAL	A	818	46.982	11.994	4.926	1.00 28.64	C
ATOM	4228	O	VAL	A	818	46.704	11.661	3.763	1.00 27.81	O
ATOM	4229	N	GLY	A	819	47.793	11.275	5.699	1.00 27.84	N
ATOM	4231	CA	GLY	A	819	48.493	10.127	5.168	1.00 27.87	C
ATOM	4234	C	GLY	A	819	47.525	9.014	4.788	1.00 27.91	C
ATOM	4235	O	GLY	A	819	47.700	8.314	3.792	1.00 27.09	O
ATOM	4236	N	PHE	A	820	46.505	8.848	5.606	1.00 27.82	N
ATOM	4238	CA	PHE	A	820	45.521	7.812	5.419	1.00 28.51	C
ATOM	4240	CB	PHE	A	820	44.730	7.606	6.720	1.00 29.12	C
ATOM	4243	CG	PHE	A	820	43.822	6.403	6.710	1.00 30.74	C
ATOM	4244	CD1	PHE	A	820	44.319	5.143	7.000	1.00 33.05	C
ATOM	4246	CE1	PHE	A	820	43.486	4.028	7.004	1.00 33.70	C
ATOM	4248	CZ	PHE	A	820	42.149	4.170	6.733	1.00 34.30	C
ATOM	4250	CE2	PHE	A	820	41.622	5.438	6.498	1.00 35.70	C
ATOM	4252	CD2	PHE	A	820	42.471	6.547	6.470	1.00 34.48	C
ATOM	4254	C	PHE	A	820	44.627	8.161	4.231	1.00 28.44	C
ATOM	4255	O	PHE	A	820	44.289	7.300	3.437	1.00 29.38	O
ATOM	4256	N	ILE	A	821	44.288	9.425	4.075	1.00 28.63	N
ATOM	4258	CA	ILE	A	821	43.512	9.861	2.914	1.00 29.24	C
ATOM	4260	CB	ILE	A	821	43.138	11.354	3.044	1.00 29.40	C
ATOM	4262	CG1	ILE	A	821	42.064	11.518	4.136	1.00 30.02	C
ATOM	4265	CD1	ILE	A	821	41.748	12.975	4.560	1.00 31.19	C
ATOM	4269	CG2	ILE	A	821	42.656	11.908	1.709	1.00 29.92	C
ATOM	4273	C	ILE	A	821	44.266	9.592	1.618	1.00 29.05	C

ATOM	4274	O	ILE	A	821	43.742	8.990	0.711	1.00	28.50	O
ATOM	4275	N	ASP	A	822	45.520	10.008	1.569	1.00	29.41	N
ATOM	4277	CA	ASP	A	822	46.356	9.821	0.390	1.00	29.40	C
ATOM	4279	CB	ASP	A	822	47.694	10.533	0.583	1.00	30.20	C
ATOM	4282	CG	ASP	A	822	47.578	12.026	0.409	1.00	30.26	C
ATOM	4283	OD1	ASP	A	822	46.456	12.484	0.103	1.00	33.07	O
ATOM	4284	OD2	ASP	A	822	48.535	12.817	0.581	1.00	31.23	O
ATOM	4285	C	ASP	A	822	46.604	8.377	0.037	1.00	29.48	C
ATOM	4286	O	ASP	A	822	46.465	7.996	-1.110	1.00	30.16	O
ATOM	4287	N	ALA	A	823	46.947	7.557	1.021	1.00	29.03	N
ATOM	4289	CA	ALA	A	823	47.291	6.173	0.780	1.00	28.80	C
ATOM	4291	CB	ALA	A	823	48.068	5.616	1.972	1.00	28.84	C
ATOM	4295	C	ALA	A	823	46.087	5.270	0.480	1.00	29.37	C
ATOM	4296	O	ALA	A	823	46.188	4.359	-0.334	1.00	28.45	O
ATOM	4297	N	ILE	A	824	44.968	5.514	1.157	1.00	30.57	N
ATOM	4299	CA	ILE	A	824	43.870	4.549	1.248	1.00	31.06	C
ATOM	4301	CB	ILE	A	824	43.657	4.173	2.744	1.00	31.11	C
ATOM	4303	CG1	ILE	A	824	44.880	3.451	3.316	1.00	32.33	C
ATOM	4306	CD1	ILE	A	824	45.376	2.247	2.543	1.00	33.90	C
ATOM	4310	CG2	ILE	A	824	42.377	3.424	2.948	1.00	31.53	C
ATOM	4314	C	ILE	A	824	42.546	5.075	0.675	1.00	31.38	C
ATOM	4315	O	ILE	A	824	41.849	4.343	-0.013	1.00	31.82	O
ATOM	4316	N	CYS	A	825	42.188	6.319	0.977	1.00	31.69	N
ATOM	4318	CA	CYS	A	825	40.791	6.784	0.810	1.00	32.07	C
ATOM	4320	CB	CYS	A	825	40.430	7.766	1.923	1.00	31.91	C
ATOM	4323	SG	CYS	A	825	40.509	7.071	3.573	1.00	33.39	S
ATOM	4324	C	CYS	A	825	40.477	7.449	-0.521	1.00	32.49	C
ATOM	4325	O	CYS	A	825	39.491	7.110	-1.186	1.00	32.44	O
ATOM	4326	N	LEU	A	826	41.305	8.411	-0.887	1.00	32.14	N
ATOM	4328	CA	LEU	A	826	41.068	9.228	-2.060	1.00	33.82	C
ATOM	4330	CB	LEU	A	826	42.279	10.141	-2.258	1.00	34.35	C
ATOM	4333	CG	LEU	A	826	42.094	11.541	-2.771	1.00	36.34	C
ATOM	4335	CD1	LEU	A	826	41.169	12.322	-1.846	1.00	37.86	C
ATOM	4339	CD2	LEU	A	826	43.498	12.193	-2.926	1.00	36.47	C
ATOM	4343	C	LEU	A	826	40.796	8.398	-3.330	1.00	33.83	C
ATOM	4344	O	LEU	A	826	39.797	8.594	-4.012	1.00	33.90	O
ATOM	4345	N	GLN	A	827	41.676	7.453	-3.629	1.00	33.95	N
ATOM	4347	CA	GLN	A	827	41.520	6.625	-4.814	1.00	34.31	C
ATOM	4349	CB	GLN	A	827	42.734	5.712	-5.013	1.00	34.21	C
ATOM	4352	CG	GLN	A	827	43.454	5.940	-6.309	1.00	37.79	C
ATOM	4355	CD	GLN	A	827	44.483	4.845	-6.624	1.00	39.98	C
ATOM	4356	OE1	GLN	A	827	45.145	4.330	-5.722	1.00	39.97	O
ATOM	4357	NE2	GLN	A	827	44.598	4.492	-7.891	1.00	37.98	N
ATOM	4360	C	GLN	A	827	40.237	5.791	-4.799	1.00	33.59	C
ATOM	4361	O	GLN	A	827	39.624	5.592	-5.826	1.00	33.68	O
ATOM	4362	N	LEU	A	828	39.857	5.276	-3.644	1.00	33.46	N
ATOM	4364	CA	LEU	A	828	38.632	4.495	-3.529	1.00	33.05	C
ATOM	4366	CB	LEU	A	828	38.542	3.885	-2.143	1.00	33.17	C
ATOM	4369	CG	LEU	A	828	37.237	3.166	-1.802	1.00	35.09	C
ATOM	4371	CD1	LEU	A	828	36.965	2.045	-2.823	1.00	37.34	C
ATOM	4375	CD2	LEU	A	828	37.321	2.625	-0.429	1.00	34.66	C
ATOM	4379	C	LEU	A	828	37.356	5.313	-3.849	1.00	32.72	C
ATOM	4380	O	LEU	A	828	36.474	4.839	-4.560	1.00	31.03	O
ATOM	4381	N	TYR	A	829	37.250	6.521	-3.292	1.00	32.51	N
ATOM	4383	CA	TYR	A	829	36.064	7.347	-3.485	1.00	31.96	C
ATOM	4385	CB	TYR	A	829	35.979	8.420	-2.386	1.00	31.66	C
ATOM	4388	CG	TYR	A	829	35.683	7.843	-1.009	1.00	32.31	C
ATOM	4389	CD1	TYR	A	829	36.587	7.999	0.056	1.00	30.90	C
ATOM	4391	CE1	TYR	A	829	36.342	7.453	1.282	1.00	32.79	C
ATOM	4393	CZ	TYR	A	829	35.174	6.718	1.492	1.00	33.50	C
ATOM	4394	OH	TYR	A	829	34.918	6.191	2.724	1.00	29.58	O
ATOM	4396	CE2	TYR	A	829	34.259	6.547	0.470	1.00	32.83	C
ATOM	4398	CD2	TYR	A	829	34.526	7.101	-0.780	1.00	32.09	C
ATOM	4400	C	TYR	A	829	36.061	7.926	-4.926	1.00	32.09	C
ATOM	4401	O	TYR	A	829	35.013	8.092	-5.507	1.00	31.90	O
ATOM	4402	N	GLU	A	830	37.237	8.178	-5.505	1.00	32.32	N
ATOM	4404	CA	GLU	A	830	37.357	8.521	-6.938	1.00	33.53	C

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ATOM   4406  CB  GLU A 830      38.813   8.832  -7.374  1.00 33.92           C
ATOM   4409  CG  GLU A 830      39.422  10.137  -6.848  1.00 36.91           C
ATOM   4412  CD  GLU A 830      40.952  10.292  -7.084  1.00 42.60           C
ATOM   4413  OE1 GLU A 830      41.515  11.357  -6.706  1.00 46.07           O
ATOM   4414  OE2 GLU A 830      41.626   9.381  -7.636  1.00 45.22           O
ATOM   4415  C   GLU A 830      36.817   7.382  -7.788  1.00 33.38           C
ATOM   4416  O   GLU A 830      35.994   7.600  -8.691  1.00 32.83           O
ATOM   4417  N   ALA A 831      37.261   6.165  -7.487  1.00 33.32           N
ATOM   4419  CA  ALA A 831      36.801   4.983  -8.232  1.00 33.85           C
ATOM   4421  CB  ALA A 831      37.571   3.712  -7.831  1.00 33.75           C
ATOM   4425  C   ALA A 831      35.301   4.760  -8.066  1.00 33.80           C
ATOM   4426  O   ALA A 831      34.634   4.435  -9.027  1.00 34.04           O
ATOM   4427  N   LEU A 832      34.778   4.935  -6.856  1.00 33.40           N
ATOM   4429  CA  LEU A 832      33.353   4.752  -6.619  1.00 33.24           C
ATOM   4431  CB  LEU A 832      33.023   4.977  -5.146  1.00 33.06           C
ATOM   4434  CG  LEU A 832      31.587   4.714  -4.691  1.00 33.84           C
ATOM   4436  CD1 LEU A 832      31.165   3.309  -5.086  1.00 33.85           C
ATOM   4440  CD2 LEU A 832      31.420   4.916  -3.160  1.00 36.52           C
ATOM   4444  C   LEU A 832      32.530   5.734  -7.451  1.00 32.81           C
ATOM   4445  O   LEU A 832      31.456   5.410  -7.942  1.00 32.89           O
ATOM   4446  N   THR A 833      33.030   6.952  -7.565  1.00 32.75           N
ATOM   4448  CA  THR A 833      32.333   8.020  -8.272  1.00 32.75           C
ATOM   4450  CB  THR A 833      33.055   9.337  -7.978  1.00 33.07           C
ATOM   4452  OG1 THR A 833      32.827   9.686  -6.599  1.00 33.82           O
ATOM   4454  CG2 THR A 833      32.504  10.472  -8.751  1.00 33.21           C
ATOM   4458  C   THR A 833      32.228   7.728  -9.772  1.00 32.38           C
ATOM   4459  O   THR A 833      31.234   8.077 -10.405  1.00 31.86           O
ATOM   4460  N   HIS A 834      33.233   7.058 -10.321  1.00 32.05           N
ATOM   4462  CA  HIS A 834      33.201   6.605 -11.700  1.00 32.54           C
ATOM   4464  CB  HIS A 834      34.578   6.085 -12.143  1.00 32.85           C
ATOM   4467  CG  HIS A 834      35.565   7.170 -12.448  1.00 34.19           C
ATOM   4468  ND1 HIS A 834      35.416   8.036 -13.515  1.00 36.12           N
ATOM   4470  CE1 HIS A 834      36.436   8.876 -13.540  1.00 36.63           C
ATOM   4472  NE2 HIS A 834      37.243   8.587 -12.532  1.00 36.17           N
ATOM   4474  CD2 HIS A 834      36.725   7.519 -11.838  1.00 35.04           C
ATOM   4476  C   HIS A 834      32.128   5.529 -11.913  1.00 32.37           C
ATOM   4477  O   HIS A 834      31.503   5.484 -12.958  1.00 30.88           O
ATOM   4478  N   VAL A 835      31.921   4.668 -10.922  1.00 32.30           N
ATOM   4480  CA  VAL A 835      30.862   3.658 -11.018  1.00 32.46           C
ATOM   4482  CB  VAL A 835      31.036   2.566  -9.940  1.00 32.60           C
ATOM   4484  CG1 VAL A 835      29.819   1.639  -9.907  1.00 33.06           C
ATOM   4488  CG2 VAL A 835      32.313   1.764 -10.185  1.00 32.37           C
ATOM   4492  C   VAL A 835      29.474   4.293 -10.870  1.00 32.47           C
ATOM   4493  O   VAL A 835      28.527   3.897 -11.548  1.00 32.48           O
ATOM   4494  N   SER A 836      29.359   5.241  -9.935  1.00 32.44           N
ATOM   4496  CA  SER A 836      28.134   5.995  -9.720  1.00 32.19           C
ATOM   4498  CB  SER A 836      27.294   5.378  -8.628  1.00 32.33           C
ATOM   4501  OG  SER A 836      26.015   5.987  -8.624  1.00 34.05           O
ATOM   4503  C   SER A 836      28.453   7.431  -9.366  1.00 31.80           C
ATOM   4504  O   SER A 836      28.979   7.724  -8.286  1.00 31.10           O
ATOM   4505  N   GLU A 837      28.132   8.331 -10.288  1.00 31.31           N
ATOM   4507  CA  GLU A 837      28.449   9.737 -10.127  1.00 31.78           C
ATOM   4509  CB  GLU A 837      28.084  10.510 -11.401  1.00 32.41           C
ATOM   4512  CG  GLU A 837      28.646  11.930 -11.471  1.00 36.07           C
ATOM   4515  CD  GLU A 837      30.173  11.984 -11.392  1.00 41.36           C
ATOM   4516  OE1 GLU A 837      30.848  11.020 -11.858  1.00 43.72           O
ATOM   4517  OE2 GLU A 837      30.700  13.002 -10.869  1.00 45.04           O
ATOM   4518  C   GLU A 837      27.746  10.346  -8.910  1.00 30.90           C
ATOM   4519  O   GLU A 837      28.237  11.318  -8.344  1.00 30.45           O
ATOM   4520  N   ASP A 838      26.615   9.771  -8.509  1.00 30.19           N
ATOM   4522  CA  ASP A 838      25.896  10.213  -7.307  1.00 30.79           C
ATOM   4524  CB  ASP A 838      24.494   9.626  -7.312  1.00 30.45           C
ATOM   4527  CG  ASP A 838      23.733   9.988  -8.566  1.00 33.30           C
ATOM   4528  OD1 ASP A 838      23.271  11.135  -8.642  1.00 30.59           O
ATOM   4529  OD2 ASP A 838      23.594   9.204  -9.539  1.00 37.40           O
ATOM   4530  C   ASP A 838      26.595   9.856  -5.964  1.00 30.92           C
ATOM   4531  O   ASP A 838      26.143  10.267  -4.897  1.00 30.59           O
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ATOM	4532	N	CYS	A	839	27.662	9.074	-6.012	1.00	30.74	N
ATOM	4534	CA	CYS	A	839	28.507	8.897	-4.824	1.00	31.60	C
ATOM	4536	CB	CYS	A	839	29.171	7.532	-4.846	1.00	31.36	C
ATOM	4539	SG	CYS	A	839	28.024	6.171	-4.545	1.00	32.85	S
ATOM	4540	C	CYS	A	839	29.562	10.003	-4.724	1.00	31.85	C
ATOM	4541	O	CYS	A	839	30.388	9.989	-3.819	1.00	31.38	O
ATOM	4542	N	PHE	A	840	29.491	10.986	-5.631	1.00	32.36	N
ATOM	4544	CA	PHE	A	840	30.387	12.136	-5.597	1.00	32.57	C
ATOM	4546	CB	PHE	A	840	29.990	13.214	-6.615	1.00	32.56	C
ATOM	4549	CG	PHE	A	840	30.852	14.450	-6.531	1.00	33.86	C
ATOM	4550	CD1	PHE	A	840	32.178	14.413	-6.946	1.00	34.98	C
ATOM	4552	CE1	PHE	A	840	32.988	15.529	-6.838	1.00	34.46	C
ATOM	4554	CZ	PHE	A	840	32.480	16.693	-6.291	1.00	34.57	C
ATOM	4556	CE2	PHE	A	840	31.165	16.741	-5.860	1.00	34.02	C
ATOM	4558	CD2	PHE	A	840	30.359	15.627	-5.978	1.00	34.40	C
ATOM	4560	C	PHE	A	840	30.548	12.796	-4.211	1.00	32.63	C
ATOM	4561	O	PHE	A	840	31.668	13.162	-3.875	1.00	33.01	O
ATOM	4562	N	PRO	A	841	29.491	12.988	-3.412	1.00	32.31	N
ATOM	4563	CA	PRO	A	841	29.676	13.675	-2.124	1.00	32.29	C
ATOM	4565	CB	PRO	A	841	28.258	13.741	-1.530	1.00	32.33	C
ATOM	4568	CG	PRO	A	841	27.349	13.598	-2.712	1.00	32.91	C
ATOM	4571	CD	PRO	A	841	28.075	12.639	-3.630	1.00	32.72	C
ATOM	4574	C	PRO	A	841	30.657	12.981	-1.180	1.00	31.80	C
ATOM	4575	O	PRO	A	841	31.259	13.662	-0.373	1.00	32.29	O
ATOM	4576	N	LEU	A	842	30.840	11.675	-1.294	1.00	31.97	N
ATOM	4578	CA	LEU	A	842	31.839	10.973	-0.478	1.00	32.24	C
ATOM	4580	CB	LEU	A	842	31.730	9.457	-0.626	1.00	31.80	C
ATOM	4583	CG	LEU	A	842	30.421	8.832	-0.137	1.00	34.69	C
ATOM	4585	CD1	LEU	A	842	30.347	7.408	-0.600	1.00	37.43	C
ATOM	4589	CD2	LEU	A	842	30.248	8.889	1.388	1.00	36.33	C
ATOM	4593	C	LEU	A	842	33.240	11.442	-0.847	1.00	31.96	C
ATOM	4594	O	LEU	A	842	34.037	11.751	0.023	1.00	32.60	O
ATOM	4595	N	LEU	A	843	33.531	11.497	-2.140	1.00	31.51	N
ATOM	4597	CA	LEU	A	843	34.797	12.014	-2.618	1.00	31.36	C
ATOM	4599	CB	LEU	A	843	34.873	11.858	-4.150	1.00	31.83	C
ATOM	4602	CG	LEU	A	843	36.051	12.457	-4.915	1.00	31.94	C
ATOM	4604	CD1	LEU	A	843	37.368	11.874	-4.389	1.00	32.96	C
ATOM	4608	CD2	LEU	A	843	35.880	12.174	-6.413	1.00	33.16	C
ATOM	4612	C	LEU	A	843	34.992	13.486	-2.243	1.00	30.91	C
ATOM	4613	O	LEU	A	843	36.033	13.876	-1.752	1.00	30.62	O
ATOM	4614	N	ASP	A	844	33.989	14.306	-2.501	1.00	31.32	N
ATOM	4616	CA	ASP	A	844	34.071	15.736	-2.204	1.00	31.76	C
ATOM	4618	CB	ASP	A	844	32.764	16.414	-2.608	1.00	32.08	C
ATOM	4621	CG	ASP	A	844	32.871	17.923	-2.647	1.00	32.63	C
ATOM	4622	OD1	ASP	A	844	33.895	18.463	-3.106	1.00	35.84	O
ATOM	4623	OD2	ASP	A	844	31.970	18.655	-2.232	1.00	33.70	O
ATOM	4624	C	ASP	A	844	34.385	15.959	-0.708	1.00	31.46	C
ATOM	4625	O	ASP	A	844	35.266	16.740	-0.362	1.00	31.17	O
ATOM	4626	N	GLY	A	845	33.694	15.229	0.163	1.00	31.38	N
ATOM	4628	CA	GLY	A	845	33.878	15.355	1.600	1.00	31.48	C
ATOM	4631	C	GLY	A	845	35.266	14.898	2.028	1.00	31.50	C
ATOM	4632	O	GLY	A	845	35.919	15.545	2.852	1.00	31.47	O
ATOM	4633	N	CYS	A	846	35.731	13.790	1.457	1.00	31.20	N
ATOM	4635	CA	CYS	A	846	37.101	13.360	1.667	1.00	30.97	C
ATOM	4637	CB	CYS	A	846	37.340	12.059	0.908	1.00	31.85	C
ATOM	4640	SG	CYS	A	846	38.940	11.316	1.228	1.00	32.79	S
ATOM	4641	C	CYS	A	846	38.132	14.435	1.253	1.00	30.87	C
ATOM	4642	O	CYS	A	846	39.072	14.739	1.992	1.00	29.64	O
ATOM	4643	N	ARG	A	847	37.958	15.003	0.067	1.00	30.89	N
ATOM	4645	CA	ARG	A	847	38.833	16.059	-0.419	1.00	30.94	C
ATOM	4647	CB	ARG	A	847	38.417	16.506	-1.823	1.00	31.78	C
ATOM	4650	CG	ARG	A	847	38.945	15.636	-2.956	1.00	33.28	C
ATOM	4653	CD	ARG	A	847	38.473	16.108	-4.324	1.00	34.97	C
ATOM	4656	NE	ARG	A	847	38.881	15.180	-5.380	1.00	37.42	N
ATOM	4658	CZ	ARG	A	847	38.319	15.114	-6.588	1.00	38.64	C
ATOM	4659	NH1	ARG	A	847	37.290	15.882	-6.915	1.00	37.39	N
ATOM	4662	NH2	ARG	A	847	38.782	14.241	-7.472	1.00	40.75	N

ATOM	4665	C	ARG	A	847	38.838	17.291	0.489	1.00	30.67	C
ATOM	4666	O	ARG	A	847	39.898	17.891	0.708	1.00	30.13	O
ATOM	4667	N	LYS	A	848	37.669	17.672	1.010	1.00	30.00	N
ATOM	4669	CA	LYS	A	848	37.572	18.873	1.840	1.00	30.06	C
ATOM	4671	CB	LYS	A	848	36.119	19.316	2.035	1.00	30.40	C
ATOM	4674	CG	LYS	A	848	35.493	19.974	0.810	1.00	32.67	C
ATOM	4677	CD	LYS	A	848	33.969	20.151	1.034	1.00	35.48	C
ATOM	4680	CE	LYS	A	848	33.273	20.910	-0.099	1.00	35.58	C
ATOM	4683	NZ	LYS	A	848	31.803	20.591	-0.131	1.00	36.69	N
ATOM	4687	C	LYS	A	848	38.244	18.652	3.185	1.00	29.16	C
ATOM	4688	O	LYS	A	848	38.875	19.561	3.694	1.00	28.25	O
ATOM	4689	N	ASN	A	849	38.131	17.434	3.728	1.00	29.15	N
ATOM	4691	CA	ASN	A	849	38.786	17.075	5.002	1.00	29.60	C
ATOM	4693	CB	ASN	A	849	38.258	15.748	5.583	1.00	29.71	C
ATOM	4696	CG	ASN	A	849	36.826	15.867	6.117	1.00	28.85	C
ATOM	4697	OD1	ASN	A	849	36.402	16.925	6.530	1.00	32.39	O
ATOM	4698	ND2	ASN	A	849	36.093	14.779	6.097	1.00	28.94	N
ATOM	4701	C	ASN	A	849	40.298	17.023	4.862	1.00	29.97	C
ATOM	4702	O	ASN	A	849	41.024	17.445	5.777	1.00	30.10	O
ATOM	4703	N	ARG	A	850	40.783	16.550	3.710	1.00	30.30	N
ATOM	4705	CA	ARG	A	850	42.224	16.548	3.441	1.00	30.06	C
ATOM	4707	CB	ARG	A	850	42.525	15.868	2.103	1.00	30.76	C
ATOM	4710	CG	ARG	A	850	43.997	15.480	1.922	1.00	30.45	C
ATOM	4713	CD	ARG	A	850	44.536	15.663	0.516	1.00	31.23	C
ATOM	4716	NE	ARG	A	850	45.953	15.294	0.431	1.00	31.15	N
ATOM	4718	CZ	ARG	A	850	46.981	16.122	0.656	1.00	31.68	C
ATOM	4719	NH1	ARG	A	850	48.226	15.661	0.572	1.00	31.87	N
ATOM	4722	NH2	ARG	A	850	46.796	17.395	0.962	1.00	32.03	N
ATOM	4725	C	ARG	A	850	42.765	17.979	3.457	1.00	30.37	C
ATOM	4726	O	ARG	A	850	43.801	18.245	4.058	1.00	29.83	O
ATOM	4727	N	GLN	A	851	42.045	18.895	2.812	1.00	31.08	N
ATOM	4729	CA	GLN	A	851	42.403	20.317	2.806	1.00	32.13	C
ATOM	4731	CB	GLN	A	851	41.380	21.178	2.035	1.00	32.54	C
ATOM	4734	CG	GLN	A	851	41.601	21.299	0.549	1.00	35.11	C
ATOM	4737	CD	GLN	A	851	40.739	22.400	-0.088	1.00	37.85	C
ATOM	4738	OE1	GLN	A	851	41.250	23.451	-0.507	1.00	38.21	O
ATOM	4739	NE2	GLN	A	851	39.425	22.157	-0.155	1.00	39.86	N
ATOM	4742	C	GLN	A	851	42.488	20.837	4.238	1.00	31.69	C
ATOM	4743	O	GLN	A	851	43.473	21.471	4.608	1.00	31.56	O
ATOM	4744	N	LYS	A	852	41.458	20.548	5.034	1.00	31.11	N
ATOM	4746	CA	LYS	A	852	41.385	21.025	6.412	1.00	31.32	C
ATOM	4748	CB	LYS	A	852	39.988	20.757	7.010	1.00	31.63	C
ATOM	4751	CG	LYS	A	852	38.850	21.660	6.454	1.00	32.95	C
ATOM	4754	CD	LYS	A	852	38.794	23.023	7.177	1.00	35.29	C
ATOM	4757	CE	LYS	A	852	37.794	24.003	6.545	1.00	36.53	C
ATOM	4760	NZ	LYS	A	852	38.394	25.348	6.227	1.00	38.20	N
ATOM	4764	C	LYS	A	852	42.509	20.444	7.299	1.00	30.77	C
ATOM	4765	O	LYS	A	852	43.193	21.190	8.003	1.00	31.25	O
ATOM	4766	N	TRP	A	853	42.732	19.135	7.238	1.00	30.53	N
ATOM	4768	CA	TRP	A	853	43.836	18.505	7.981	1.00	30.28	C
ATOM	4770	CB	TRP	A	853	43.772	16.973	7.904	1.00	30.25	C
ATOM	4773	CG	TRP	A	853	42.685	16.327	8.734	1.00	29.17	C
ATOM	4774	CD1	TRP	A	853	41.682	15.521	8.277	1.00	29.72	C
ATOM	4776	NE1	TRP	A	853	40.895	15.092	9.318	1.00	27.75	N
ATOM	4778	CE2	TRP	A	853	41.394	15.608	10.489	1.00	29.96	C
ATOM	4779	CD2	TRP	A	853	42.519	16.395	10.158	1.00	29.49	C
ATOM	4780	CE3	TRP	A	853	43.196	17.053	11.192	1.00	29.21	C
ATOM	4782	CZ3	TRP	A	853	42.739	16.904	12.502	1.00	30.37	C
ATOM	4784	CH2	TRP	A	853	41.628	16.120	12.798	1.00	28.68	C
ATOM	4786	CZ2	TRP	A	853	40.930	15.475	11.810	1.00	30.57	C
ATOM	4788	C	TRP	A	853	45.213	18.967	7.508	1.00	30.57	C
ATOM	4789	O	TRP	A	853	46.093	19.176	8.332	1.00	29.97	O
ATOM	4790	N	GLN	A	854	45.405	19.133	6.195	1.00	31.37	N
ATOM	4792	CA	GLN	A	854	46.696	19.613	5.660	1.00	32.06	C
ATOM	4794	CB	GLN	A	854	46.722	19.603	4.131	1.00	32.60	C
ATOM	4797	CG	GLN	A	854	48.091	20.008	3.493	1.00	34.51	C
ATOM	4800	CD	GLN	A	854	49.224	19.077	3.896	1.00	35.78	C

ATOM	4801	OE1	GLN	A	854	48.990	17.924	4.265	1.00	35.86	O
ATOM	4802	NE2	GLN	A	854	50.450	19.580	3.842	1.00	37.93	N
ATOM	4805	C	GLN	A	854	47.025	21.017	6.144	1.00	32.21	C
ATOM	4806	O	GLN	A	854	48.164	21.287	6.460	1.00	31.44	O
ATOM	4807	N	ALA	A	855	46.019	21.891	6.207	1.00	32.96	N
ATOM	4809	CA	ALA	A	855	46.198	23.258	6.710	1.00	33.54	C
ATOM	4811	CB	ALA	A	855	44.943	24.112	6.487	1.00	33.09	C
ATOM	4815	C	ALA	A	855	46.553	23.222	8.185	1.00	34.08	C
ATOM	4816	O	ALA	A	855	47.357	24.019	8.641	1.00	34.67	O
ATOM	4817	N	LEU	A	856	45.956	22.291	8.921	1.00	34.47	N
ATOM	4819	CA	LEU	A	856	46.308	22.066	10.318	1.00	35.05	C
ATOM	4821	CB	LEU	A	856	45.275	21.160	10.984	1.00	35.45	C
ATOM	4824	CG	LEU	A	856	44.140	21.879	11.685	1.00	35.64	C
ATOM	4826	CD1	LEU	A	856	43.111	20.854	12.128	1.00	35.60	C
ATOM	4830	CD2	LEU	A	856	44.669	22.679	12.876	1.00	36.89	C
ATOM	4834	C	LEU	A	856	47.713	21.487	10.520	1.00	35.54	C
ATOM	4835	O	LEU	A	856	48.405	21.871	11.469	1.00	36.00	O
ATOM	4836	N	ALA	A	857	48.151	20.596	9.632	1.00	36.23	N
ATOM	4838	CA	ALA	A	857	49.512	20.032	9.707	1.00	36.86	C
ATOM	4840	CB	ALA	A	857	49.675	18.886	8.730	1.00	36.47	C
ATOM	4844	C	ALA	A	857	50.583	21.096	9.452	1.00	38.02	C
ATOM	4845	O	ALA	A	857	51.708	20.990	9.947	1.00	38.57	O
ATOM	4846	N	GLU	A	858	50.220	22.120	8.689	1.00	39.06	N
ATOM	4848	CA	GLU	A	858	51.147	23.160	8.274	1.00	39.95	C
ATOM	4850	CB	GLU	A	858	50.714	23.720	6.921	1.00	40.18	C
ATOM	4853	CG	GLU	A	858	51.060	22.815	5.744	1.00	40.91	C
ATOM	4856	CD	GLU	A	858	50.697	23.429	4.408	1.00	42.60	C
ATOM	4857	OE1	GLU	A	858	50.787	24.669	4.283	1.00	45.70	O
ATOM	4858	OE2	GLU	A	858	50.337	22.685	3.475	1.00	43.19	O
ATOM	4859	C	GLU	A	858	51.198	24.263	9.317	1.00	40.65	C
ATOM	4860	O	GLU	A	858	52.258	24.808	9.616	1.00	40.62	O
ATOM	4861	N	GLN	A	859	50.034	24.584	9.870	1.00	41.69	N
ATOM	4863	CA	GLN	A	859	49.931	25.557	10.945	1.00	42.43	C
ATOM	4865	CB	GLN	A	859	48.473	25.911	11.212	1.00	42.25	C
ATOM	4868	CG	GLN	A	859	48.283	27.204	12.027	1.00	42.92	C
ATOM	4871	CD	GLN	A	859	47.100	27.140	12.973	1.00	42.72	C
ATOM	4872	OE1	GLN	A	859	46.619	26.054	13.304	1.00	42.44	O
ATOM	4873	NE2	GLN	A	859	46.620	28.307	13.404	1.00	42.81	N
ATOM	4876	C	GLN	A	859	50.582	25.026	12.218	1.00	43.48	C
ATOM	4877	O	GLN	A	859	51.049	25.817	13.037	1.00	44.43	O
ATOM	4878	N	GLN	A	860	50.640	23.702	12.374	1.00	44.48	N
ATOM	4880	CA	GLN	A	860	51.220	23.091	13.571	1.00	45.14	C
ATOM	4882	CB	GLN	A	860	50.674	21.680	13.810	1.00	45.53	C
ATOM	4885	CG	GLN	A	860	49.391	21.646	14.675	1.00	45.81	C
ATOM	4888	CD	GLN	A	860	48.904	20.223	14.929	1.00	48.17	C
ATOM	4889	OE1	GLN	A	860	47.774	20.011	15.408	1.00	49.37	O
ATOM	4890	NE2	GLN	A	860	49.747	19.239	14.593	1.00	48.49	N
ATOM	4893	C	GLN	A	860	52.741	23.080	13.502	1.00	45.80	C
ATOM	4894	O	GLN	A	860	53.399	23.165	14.541	1.00	46.30	O
ATOM	4895	N	GLU	A	861	53.298	23.014	12.290	1.00	46.36	N
ATOM	4897	CA	GLU	A	861	54.744	23.231	12.078	1.00	46.78	C
ATOM	4899	CB	GLU	A	861	55.145	22.865	10.637	1.00	46.94	C
ATOM	4902	CG	GLU	A	861	55.018	21.378	10.315	1.00	48.41	C
ATOM	4905	CD	GLU	A	861	54.889	21.091	8.816	1.00	51.01	C
ATOM	4906	OE1	GLU	A	861	55.877	21.318	8.079	1.00	51.91	O
ATOM	4907	OE2	GLU	A	861	53.799	20.630	8.368	1.00	53.39	O
ATOM	4908	C	GLU	A	861	55.187	24.682	12.406	1.00	46.87	C
ATOM	4909	O	GLU	A	861	56.380	25.003	12.379	1.00	46.87	O
ATOM	4910	N	LYS	A	862	54.213	25.542	12.722	1.00	47.14	N
ATOM	4912	CA	LYS	A	862	54.433	26.943	13.075	1.00	47.01	C
ATOM	4914	CB	LYS	A	862	55.288	27.099	14.355	1.00	47.27	C
ATOM	4917	CG	LYS	A	862	55.193	25.943	15.392	1.00	46.80	C
ATOM	4920	CD	LYS	A	862	56.571	25.256	15.615	1.00	46.48	C
ATOM	4923	CE	LYS	A	862	56.457	23.740	15.812	1.00	46.64	C
ATOM	4926	NZ	LYS	A	862	57.811	23.103	15.770	1.00	47.11	N
ATOM	4930	C	LYS	A	862	55.078	27.670	11.893	1.00	47.28	C
ATOM	4931	O	LYS	A	862	54.900	27.261	10.741	1.00	47.16	O

ATOM	4932	ZN	ZN	A	1	34.525	-0.993	10.630	1.00	50.38	ZN
ATOM	4934	O5	CIT	L	101	49.023	1.293	-4.093	1.00	68.10	O
ATOM	4935	C6	CIT	L	101	48.308	0.359	-4.647	0.50	72.51	C
ATOM	4936	O6	CIT	L	101	47.451	-0.244	-3.887	1.00	72.89	O
ATOM	4938	C3	CIT	L	101	48.403	-0.153	-6.141	0.50	73.65	C
ATOM	4939	O7	CIT	L	101	47.133	-0.911	-6.214	1.00	72.11	O
ATOM	4941	C4	CIT	L	101	48.459	0.972	-7.359	1.00	77.19	C
ATOM	4944	C5	CIT	L	101	47.271	1.292	-8.386	1.00	79.59	C
ATOM	4945	O4	CIT	L	101	46.043	0.975	-8.301	1.00	80.25	O
ATOM	4947	O3	CIT	L	101	47.505	1.962	-9.430	1.00	81.88	O
ATOM	4948	C2	CIT	L	101	49.610	-1.212	-5.995	1.00	72.14	C
ATOM	4951	C1	CIT	L	101	49.294	-2.684	-6.267	1.00	74.60	C
ATOM	4952	O1	CIT	L	101	49.594	-3.235	-7.342	1.00	77.97	O
ATOM	4953	O2	CIT	L	101	48.717	-3.440	-5.450	1.00	79.07	O
ATOM	4955	O	HOH	W	1	48.207	0.000	9.377	0.50	26.55	O
ATOM	4958	O	HOH	W	2	48.206	0.002	15.548	0.50	32.28	O
ATOM	4961	O	HOH	W	3	34.289	5.273	9.154	1.00	43.88	O
ATOM	4964	O	HOH	W	4	49.048	8.267	7.910	1.00	45.59	O
ATOM	4967	O	HOH	W	5	28.822	-0.801	21.427	1.00	49.21	O
ATOM	4970	O	HOH	W	6	43.739	-6.442	7.412	1.00	42.96	O
ATOM	4973	O	HOH	W	7	18.296	-16.040	10.216	1.00	47.23	O
ATOM	4976	O	HOH	W	8	32.430	5.453	12.380	1.00	48.00	O
ATOM	4979	O	HOH	W	9	24.183	-11.250	12.977	1.00	53.40	O
ATOM	4982	O	HOH	W	10	33.088	-13.347	14.733	1.00	50.13	O
ATOM	4985	O	HOH	W	11	16.672	10.547	4.776	1.00	58.12	O
ATOM	4988	O	HOH	W	12	41.666	-3.613	8.704	1.00	43.31	O
ATOM	4991	O	HOH	W	13	50.372	16.058	12.308	1.00	55.26	O
ATOM	4994	O	HOH	W	14	38.665	-2.779	3.708	1.00	39.85	O
ATOM	4997	O	HOH	W	15	45.675	-12.816	6.127	1.00	53.14	O
ATOM	5000	O	HOH	W	16	34.796	5.945	18.234	1.00	65.49	O
ATOM	5003	O	HOH	W	17	39.086	-6.542	-1.130	1.00	50.00	O
ATOM	5006	O	HOH	W	18	10.303	-3.731	3.425	1.00	53.09	O
ATOM	5009	O	HOH	W	19	31.249	-7.203	22.941	1.00	66.27	O
ATOM	5012	O	HOH	W	20	26.985	8.278	5.295	1.00	61.86	O
ATOM	5015	O	HOH	W	21	44.919	-5.862	-7.194	1.00	52.06	O
ATOM	5018	O	HOH	W	22	32.887	-9.277	-1.547	1.00	51.46	O
ATOM	5021	O	HOH	W	23	44.118	6.614	-2.096	1.00	51.08	O
ATOM	5024	O	HOH	W	24	38.266	9.274	10.146	1.00	49.52	O
ATOM	5027	O	HOH	W	25	42.046	4.283	16.840	1.00	64.91	O
ATOM	5030	O	HOH	W	26	20.815	-4.177	23.006	1.00	57.34	O
ATOM	5033	O	HOH	W	27	17.407	-18.805	9.260	1.00	56.41	O
ATOM	5036	O	HOH	W	28	50.908	17.436	1.757	1.00	71.67	O
ATOM	5039	O	HOH	W	29	37.973	-4.694	0.470	1.00	47.95	O
ATOM	5042	O	HOH	W	30	51.895	10.582	4.609	1.00	56.86	O
ATOM	5045	O	HOH	W	31	46.528	-3.412	-5.382	1.00	46.30	O
ATOM	5048	O	HOH	W	32	41.988	18.107	-0.911	1.00	62.92	O
ATOM	5051	O	HOH	W	33	50.668	16.518	5.962	1.00	76.18	O
ATOM	5054	O	HOH	W	34	26.830	8.551	21.466	1.00	67.61	O
ATOM	5057	O	HOH	W	35	39.963	-11.947	8.440	1.00	52.38	O
ATOM	5060	O	HOH	W	36	9.206	-2.461	8.722	1.00	67.69	O
ATOM	5063	O	HOH	W	37	27.208	7.171	-13.059	1.00	66.89	O
ATOM	5066	O	HOH	W	38	33.319	-3.136	-21.331	1.00	79.21	O
ATOM	5069	O	HOH	W	39	29.807	12.113	4.210	1.00	56.70	O
ATOM	5072	O	HOH	W	40	26.485	-2.035	22.374	1.00	59.06	O
ATOM	5075	O	HOH	W	41	13.524	-16.426	14.869	1.00	56.13	O
ATOM	5078	O	HOH	W	42	41.242	13.929	-5.574	1.00	69.30	O
ATOM	5081	O	HOH	W	43	44.000	3.616	30.031	1.00	79.86	O
ATOM	5084	O	HOH	W	44	29.770	-15.325	-3.436	1.00	72.37	O
ATOM	5087	O	HOH	W	45	55.046	-7.275	10.420	1.00	78.57	O
ATOM	5090	O	HOH	W	46	42.174	23.377	8.895	1.00	59.81	O
ATOM	5093	O	HOH	W	47	45.846	5.171	-3.429	1.00	62.43	O
ATOM	5096	O	HOH	W	48	10.721	2.483	-0.428	1.00	71.72	O
ATOM	5099	O	HOH	W	49	38.039	-8.880	-0.851	1.00	59.30	O
ATOM	5102	O	HOH	W	50	29.104	-11.816	-4.693	1.00	69.57	O
ATOM	5105	O	HOH	W	51	37.981	-1.356	6.040	1.00	52.06	O
ATOM	5108	O	HOH	W	52	38.803	-5.419	-9.275	1.00	74.64	O
ATOM	5111	O	HOH	W	53	41.112	23.777	3.772	1.00	74.75	O

ATOM	5114	O	HOH W	54	31.043	5.736	15.133	1.00	52.37	O
ATOM	5117	O	HOH W	55	25.576	-9.292	-16.079	1.00	78.39	O
ATOM	5120	O	HOH W	56	34.740	-9.401	12.971	1.00	50.11	O
ATOM	5123	O	HOH W	57	37.034	-14.827	20.525	1.00	72.86	O
ATOM	5126	O	HOH W	58	48.193	0.023	-15.539	0.50	75.94	O
ATOM	5129	O	HOH W	59	15.570	-23.396	-5.422	1.00	64.14	O
ATOM	5132	O	HOH W	60	44.168	-8.356	-7.513	1.00	64.26	O
ATOM	5135	O	HOH W	61	28.988	-3.170	-14.959	1.00	77.62	O
ATOM	5138	O	HOH W	62	20.901	-25.326	-2.234	1.00	67.25	O
ATOM	5141	O	HOH W	63	33.982	16.977	4.436	1.00	59.29	O
ATOM	5144	O	HOH W	64	37.652	17.395	18.730	1.00	70.50	O
ATOM	5147	O	HOH W	65	48.714	-1.176	12.509	1.00	55.83	O
ATOM	5150	O	HOH W	66	23.648	13.228	15.636	1.00	61.83	O
ATOM	5153	O	HOH W	67	37.930	-5.485	14.629	1.00	62.75	O
ATOM	5156	O	HOH W	68	39.361	8.113	12.281	1.00	65.09	O
ATOM	5159	O	HOH W	69	44.416	19.041	0.025	1.00	65.31	O
ATOM	5162	O	HOH W	70	31.024	-11.670	12.913	1.00	51.04	O
ATOM	5165	O	HOH W	71	30.803	-10.927	-2.260	1.00	70.95	O
ATOM	5168	O	HOH W	72	18.394	4.353	24.131	1.00	56.96	O
ATOM	5171	O	HOH W	73	40.724	-9.915	-6.592	1.00	71.31	O
ATOM	5174	O	HOH W	74	39.415	-11.451	-1.251	1.00	59.36	O
ATOM	5177	O	HOH W	75	10.729	6.380	4.422	1.00	64.07	O
ATOM	5180	O	HOH W	76	32.027	-16.657	1.416	1.00	71.19	O
ATOM	5183	O	HOH W	77	26.786	-20.606	2.339	1.00	57.23	O
ATOM	5186	O	HOH W	78	18.583	16.228	4.823	1.00	73.42	O
ATOM	5189	O	HOH W	79	27.374	7.398	8.872	1.00	62.50	O
ATOM	5192	O	HOH W	80	35.193	-16.175	10.352	1.00	73.93	O
ATOM	5195	O	HOH W	81	52.965	18.374	4.826	1.00	72.09	O
ATOM	5198	O	HOH W	82	11.094	0.000	0.113	1.00	85.89	O
ATOM	5201	O	HOH W	83	22.715	12.451	0.236	1.00	66.47	O
ATOM	5204	O	HOH W	84	29.996	6.584	8.368	1.00	57.82	O
ATOM	5207	O	HOH W	85	42.337	13.541	20.658	1.00	71.89	O
ATOM	5210	O	HOH W	86	37.358	-4.176	21.021	1.00	72.66	O
ATOM	5213	O	HOH W	87	13.285	-6.976	-6.448	1.00	67.69	O
ATOM	5216	O	HOH W	88	43.834	23.530	-1.284	1.00	77.37	O
ATOM	5219	O	HOH W	89	45.114	22.881	3.087	1.00	68.89	O
ATOM	5222	O	HOH W	90	44.237	-15.595	6.458	1.00	87.26	O

Table 2

PCR from Human Kidney QUICK-Clone cDNA (Clontech, #7112-1)

Protein in pET15S: 366 aa Mass: 42049.2 pI: 6.68

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  1 MGSSHHHHHH SSGLVPRGSH MSAAEEETRE LQSLAAAVVP SAQTLKITDF SFSDFELSDL
 61 ETALCTIRMF TDLNLVQNFQ MKHEVLCRWI LSVKKNYRKN VAYHNWRHAF NTAQCMFAAL
121 KAGKIQNKLT DLEILALLIA ALSHDLDHRG VNNSYIQRSE HPLAQLYCHS IMEHHHFDQC
181 LMILNSPGNQ ILSGLSIEEY KTTLKIIKQA ILATDLALYI KRRGEFFELI RKNQFNLEDP
241 HQKELFLAML MTACDLSAIT KPWPIQQRIA ELVATEFFDQ GDRERKELNI EPTDLMNREK
301 KNKIPSMQVG FIDAICLQLY EALTHVSEDC FPLLDGCRKN RQKWQALAEQ QEKMLINGES
361 GQAKRN
```

PDE5A-S: 5′-GTCGTAT CATATG TCAGCAGCAGAGGAAGAAAC-3′ 33 mer

PDE5A-A: 5'-TCTGCA GTCGAC AGGCCACTCAGTTCCGCTTG-3′ 32 mer

pET15S sequence (PCR product; 1070 bp)

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             t cagcagcaga
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1981 taaaattact gactttagct tcagtgactt tgagctgtct gatctggaaa cagcactgtg
2041 tacaattcgg atgtttactg acctcaacct tgtgcagaac ttccagatga aacatgaggt
2101 tctttgcaga tggattttaa gtgttaagaa gaattatcgg aagaatgttg cctatcataa
2161 ttggagacat gcctttaata cagctcagtg catgtttgct gctctaaaag caggcaaaat
2221 tcagaacaag ctgactgacc tggagatact tgcattgctg attgctgcac taagccacga
2281 tttggatcac cgtggtgtga ataactctta catacagcga agtgaacatc cacttgccca
2341 gctttactgc cattcaatca tggaacacca tcattttgac cagtgcctga tgattcttaa
2401 tagtccaggc aatcagattc tcagtggcct ctccattgaa gaatataaga ccacgttgaa
2461 aataatcaag caagctattt tagctacaga cctagcactg tacattaaga ggcgaggaga
2521 atttttttgaa cttataagaa aaaatcaatt caatttggaa gatcctcatc aaaaggagtt
2581 gtttttggca atgctgatga cagcttgtga tctttctgca attacaaaac cctggcctat
2641 tcaacaacgg atagcagaac ttgtagcaac tgaatttttt gatcaaggag acagagagag
2701 aaaagaactc aacatagaac ccactgatct aatgaacagg gagaagaaaa acaaaatccc
2761 aagtatgcaa gttgggttca tagatgccat ctgcttgcaa ctgtatgagg ccctgaccca
2821 cgtgtcagag gactgtttcc ctttgctaga tggctgcaga aagaacaggc agaaatggca
2881 ggcccttgca gaacagcagg agaagatgct gattaatggg gaaagcggcc aggccaagcg
2941 gaactgagtg gcct
GTCGACTAGAGCCTGCAGTCTCGACCATCATCATCATCATTAATAAAAGGGCGAATTCCAGCACACT
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Table 3

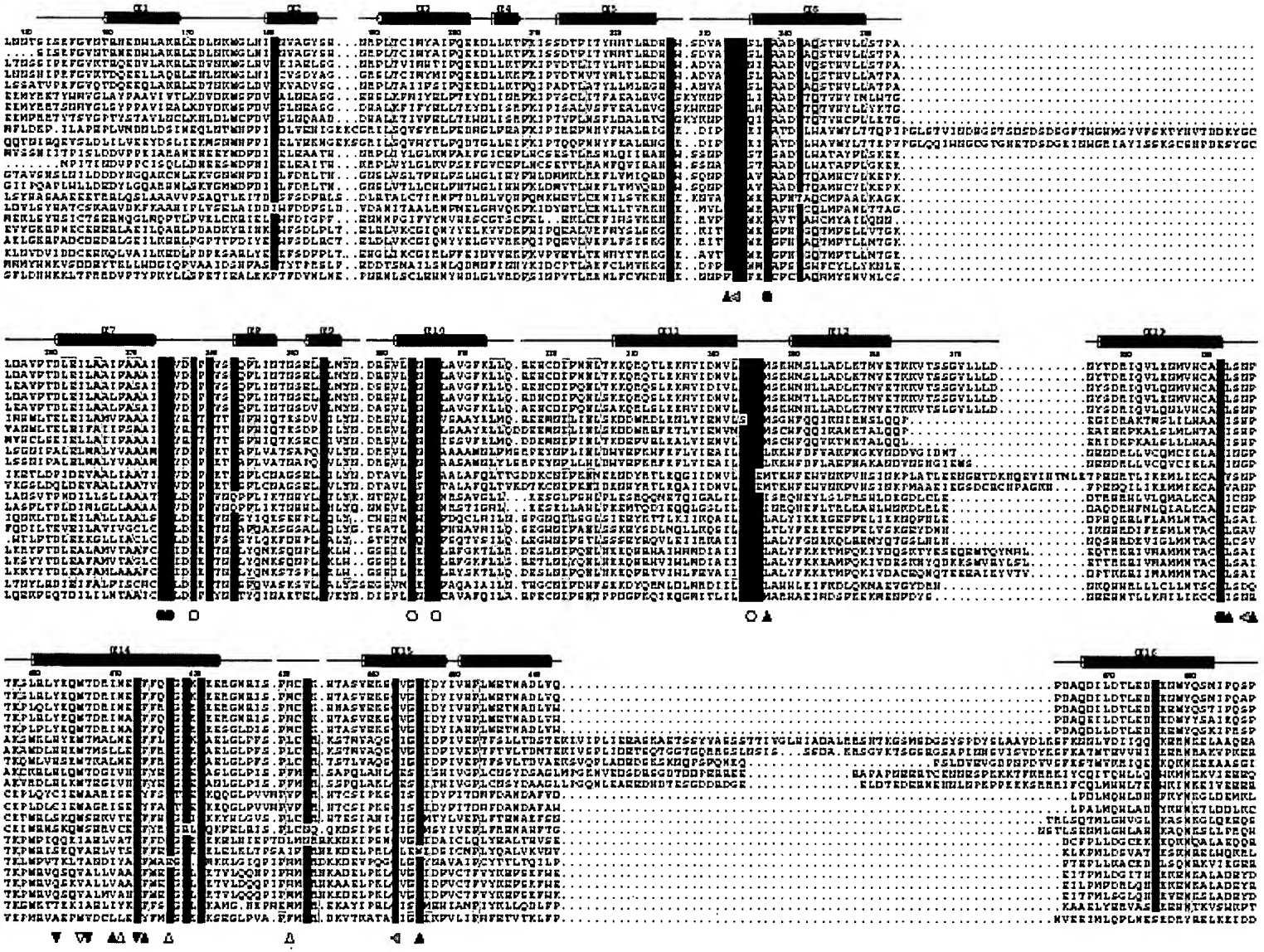

Table 4

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LOCUS       PDE5A                  3106 bp    mRNA    linear   PRI 05-NOV-2002
DEFINITION  Homo sapiens phosphodiesterase 5A, cGMP-specific (PDE5A),
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ACCESSION   NM_001083
VERSION     NM_001083.2  GI:15812210
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
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            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 3106)
  AUTHORS   Stacey,P., Rulten,S., Dapling,A. and Phillips,S.C.
  TITLE     Molecular cloning and expression of human cGMP-binding
            cGMP-specific phosphodiesterase (PDE5)
  JOURNAL   Biochem. Biophys. Res. Commun. 247 (2), 249-254 (1998)
  MEDLINE   98308101
   PUBMED   9642111
REFERENCE   2  (bases 1 to 3106)
  AUTHORS   Yanaka,N., Kotera,J., Ohtsuka,A., Akatsuka,H., Imai,Y.,
            Michibata,H., Fujishige,K., Kawai,E., Takebayashi,S., Okumura,K.
            and Omori,K.
  TITLE     Expression, structure and chromosomal localization of the human
            cGMP-binding cGMP-specific phosphodiesterase PDE5A gene
  JOURNAL   Eur. J. Biochem. 255 (2), 391-399 (1998)
  MEDLINE   98380237
   PUBMED   9716380
REFERENCE   3  (bases 1 to 3106)
  AUTHORS   Loughney,K., Hill,T.R., Florio,V.A., Uher,L., Rosman,G.J.,
            Wolda,S.L., Jones,B.A., Howard,M.L., McAllister-Lucas,L.M.,
            Sonnenburg,W.K., Francis,S.H., Corbin,J.D., Beavo,J.A. and
            Ferguson,K.
  TITLE     Isolation and characterization of cDNAs encoding PDE5A, a human
            cGMP-binding, cGMP-specific 3',5'-cyclic nucleotide
            phosphodiesterase
  JOURNAL   Gene 216 (1), 139-147 (1998)
  MEDLINE   98382582
   PUBMED   9714779
REFERENCE   4  (bases 1 to 3106)
  AUTHORS   Kotera,J., Fujishige,K., Imai,Y., Kawai,E., Michibata,H.,
            Akatsuka,H., Yanaka,N. and Omori,K.
  TITLE     Genomic origin and transcriptional regulation of two variants of
            cGMP-binding cGMP-specific phosphodiesterases
  JOURNAL   Eur. J. Biochem. 262 (3), 866-873 (1999)
  MEDLINE   99339957
   PUBMED   10411650
REFERENCE   5  (bases 1 to 3106)
  AUTHORS   Lin,C.S., Lau,A., Tu,R. and Lue,T.F.
  TITLE     Identification of three alternative first exons and an intronic
            promoter of human PDE5A gene
  JOURNAL   Biochem. Biophys. Res. Commun. 268 (2), 596-602 (2000)
  MEDLINE   20145478
   PUBMED   10679249
REFERENCE   6  (bases 1 to 3106)
  AUTHORS   Lin,C.S., Lau,A., Tu,R. and Lue,T.F.
  TITLE     Expression of three isoforms of cGMP-binding cGMP-specific
            phosphodiesterase (PDE5) in human penile cavernosum
  JOURNAL   Biochem. Biophys. Res. Commun. 268 (2), 628-635 (2000)
  MEDLINE   20145484
   PUBMED   10679255
REFERENCE   7  (bases 1 to 3106)
  AUTHORS   Lin,C.S., Chow,S., Lau,A., Tu,R. and Lue,T.F.
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  TITLE     Identification and regulation of human PDE5A gene promoter
  JOURNAL   Biochem. Biophys. Res. Commun. 280 (3), 684-692 (2001)
  MEDLINE   21092663
   PUBMED   11162575
COMMENT     REVIEWED REFSEQ: This record has been curated by NCBI staff. The
            reference sequence was derived from AF043731.1.
            On Oct 1, 2001 this sequence version replaced gi:4505666.
            Summary: This gene encodes a cGMP-binding, cGMP-specific
            phosphodiesterase, a member of the cyclic nucleotide
            phosphodiesterase family. This phosphodiesterase specifically
            hydrolyzes cGMP to 5'-GMP. It is involved in the regulation of
            intracellular concentrations of cyclic nucleotides and is important
            for smooth muscle relaxation in the cardiovascular system.
            Alternative splicing of this gene results in four transcript
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            Transcript Variant: This variant (1) encodes the longest isoform
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